JOURNAL OF DAIRY SCIENCE

Contents

Action of Butter Cultures in Butter: A Review. F. J. BABEL and B. W. HAMMER 79

A Satisfactory Method of Shipping Dairy Bull Semen Long Distances. ERIC W. SWANSON and H. A. HERMAN 143

The Accuracy of the Mojonnier Method for Estimating Milk Fat in Milk and Cream. ERNEST O. HERREID and DANA W. WHITMAN 147

The Milk and Butterfat Production Responses to Shark Liver Oil in the Ration. F. C. FOUNTAINE and D. W. BOLIN 155

A 2 x 2 Factorial Design for Double Reversal Feeding Experiments. D. M. SEATH 159

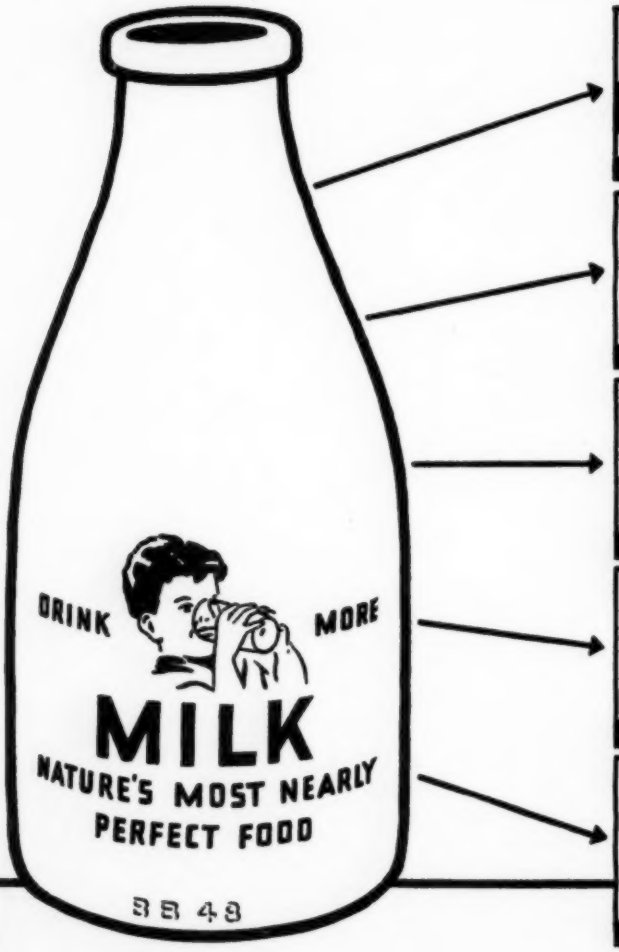
Announcement 165

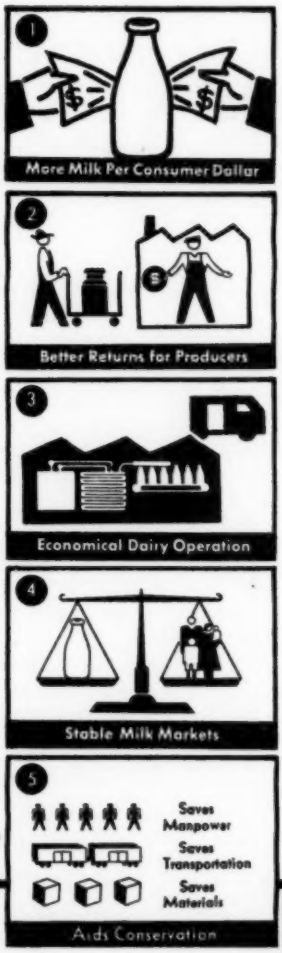
Abstracts of Literature A13

Vol. XXVII, No. 2, February, 1944

Published by the
AMERICAN DAIRY SCIENCE ASSOCIATION

GLASS BOTTLES FOR MILK MEAN:





...and Owens-Illinois Duraglas Dairy Containers mean:

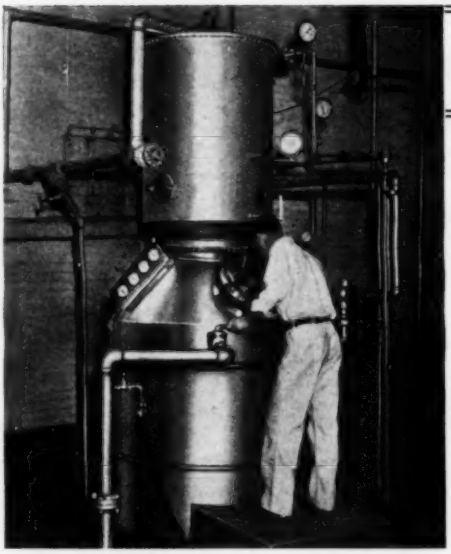
1. Quality bottles of greater strength for high trippage
2. A complete line of finishes and styles to fit your needs
3. Traditional Owens-Illinois service

OWENS ILLINOIS *Complete Dairy Service*

TOLEDO OHIO

WHERE QUALITY PREDOMINATES

Your advertisement is being read in every State and in 25 Foreign Countries



2 ITEMS OF SPECIAL INTEREST TO BUTTER MEN

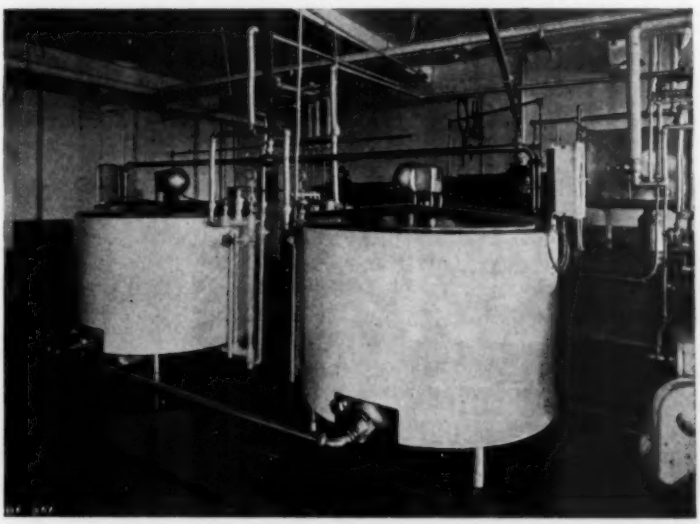
1. *Condensing Buttermilk*

The present necessity for conserving food supplies points an even greater need for condensing your buttermilk. Saving all your milk solids by using the highly successful Mojonnier Vacuum Pan is good business logic, for its operating efficiency and economy have never been equalled. Get full details now.

MOJONNIER BROS. CO. 4601 W. OHIO STREET CHICAGO 44, ILLINOIS

2. *Producing Butter of Finest Flavor and Quality*

The Mojonnier All-Purpose Cream Vat making possible all operations in *one* unit, means only *one* stickage loss. There are no stuffing boxes. Quickest cooling and heating are provided. A coil-less interior does away with tedious cleaning. Of all-welded stainless steel construction, overlapping seams, cracks and screwed joints are eliminated, making for perfect sanitation. Most important, its overall efficiency makes for a greater operating economy. Complete details on request.



Your advertisement is being read in every State and in 25 Foreign Countries

JOURNAL OF DAIRY SCIENCE

OFFICIAL ORGAN OF
AMERICAN DAIRY SCIENCE ASSOCIATION
Published at
NORTH QUEEN ST. AND MCGOVERN AVE., LANCASTER, PA.

T. S. SUTTON, Editor
Columbus, Ohio

Associate Editors

L. S. PALMER St. Paul, Minn.	C. W. LARSON Boston, Mass.	C. E. GRAY San Francisco, Cal.
E. G. HASTINGS Madison, Wis.	PAUL F. SHARP Ithaca, N. Y.	O. F. HUNZIKER Chicago, Ill.
C. A. CARY Beltsville, Md.	L. A. ROGERS Washington, D. C.	H. A. RUEHE Urbana, Ill.

Committee on Journal Management

A. A. BORLAND, *Chairman*

FORDYCE ELY — G. M. TROUT
T. S. SUTTON, *ex officio* — R. B. STOLTZ, *ex officio*

Subscriptions. Price; $6.00 per volume in North and South America; $6.50 in all other countries. Prices are net, postpaid. New subscriptions and renewals are entered to begin with the first issue of the current volume. Renewals should be made promptly to avoid a break in the series. Subscriptions should be sent to R. B. Stoltz, The Ohio State University, Columbus, Ohio.

Subscriptions for the British Isles and British Empire, except for Canada and Australia, should be ordered through our agents: Messrs. Bailliere, Tindall and Cox, 7 and 8 Henrietta Streets, Covent Garden, London, W. C. 2, England. Subscriptions for Australia should be sent to our agent: John H. Bryant, Herbert St., St. Leonards, N. S. W., Australia.

Advertising should be mailed direct to the Science Press Printing Company, N. Queen St. and McGovern Ave., Lancaster, Pennsylvania.

Post Office Notices of undeliverable copies and changes of address should be sent to R. B. Stoltz, The Ohio State University, Columbus, Ohio.

OFFICERS OF THE ASSOCIATION

A. C. DAHLBERG, President
Ithaca, New York

A. C. RAGSDALE, Vice-President
Columbia, Missouri

R. B. STOLTZ, Sec.-Treas.
Columbus, Ohio

DIRECTORS

P. H. TRACY Urbana, Illinois	W. E. PETERSEN St. Paul, Minn.	G. M. TROUT East Lansing, Mich.
C. L. BLACKMAN Columbus, Ohio	R. B. BECKER Gainesville, Fla.	J. C. KNOTT Pullman, Wash.
	H. P. DAVIS Lincoln 1, Neb.	

Entered as second-class matter April 13, 1934 at the postoffice at Lancaster, Pa., under the act of March 3, 1879.

IN THE PLANTS AND LABORATORIES OF THE VITAL DAIRY INDUSTRY...

as well as in many State conducted or approved agricultural colleges and departments, the Kimble Manual on Dairy Testing is in constant practical use.

As a measure of co-operation in the Dairy Industry's essential efforts on the Food Front, this Manual is gladly offered by the makers of

KIMBLE K BRAND DAIRY GLASSWARE

the established standard for test, control, analysis and research.

For Assurance

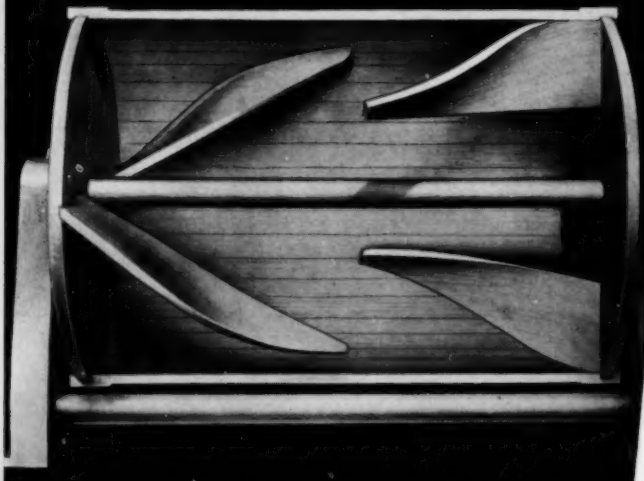
If you do not have the **THIRD EDITION** (1942) *write for copies now.*

The Visible Guarantee of Invisible Quality

KIMBLE GLASS COMPANY · · · · VINELAND, N. J.

Your advertisement is being read in every State and in 25 Foreign Countries

HOW FAST ARE YOU GETTING AWAY?



STATE OF MINNESOTA

ELMER A. BENSON
GOVERNOR

DEPARTMENT OF AGRICULTURE,
DAIRY AND FOOD
CHAS. DOWNEY Commissioner

September 15, 1937

General Dairy Equipment Co.
2920 Thirteenth Ave. S. E.
Minneapolis, Minnesota

Gentlemen:

Following are the results of tests made of each individual tub of a churning made at the Franklin Co-Operative Creamery, Minneapolis, Minnesota, on Friday, September 10th, taken at the request of the General Dairy Equipment Co., of Minneapolis. Our Mr. Torgueson witnessed the churning and took those samples from each tub and forwarded them to our laboratory here in St. Paul where the following results were ascertained by our chemist.

Tub No.	Butterfat	Fat Cent	Per Cent Water	Per Cent Salt
1	80.3	.8	14.1	2.8
2	80.3	.8	14.1	2.8
3	80.2	.8	14.3	2.8
4	80.0	.8	14.3	2.8
5	80.0	.8	14.3	2.8
6	80.0	.8	14.3	2.8
7	80.3	.8	14.2	2.8
8	80.2	.8	14.2	2.8
9	80.2	.8	14.1	2.8
10	80.3	.8	14.1	2.8
11	80.3	.8	14.2	2.8
12	80.3	.8	14.2	2.8
13	80.2	.8	14.1	2.8
14	80.2	.8	14.2	2.8
15	80.2	.8	14.2	2.8
16	80.3	.8	14.2	2.8
17	80.3	.8	14.2	2.8
18	80.2	.8	14.3	2.8
19	80.2	.8	14.3	2.8
20	80.3	.8	14.1	2.8
Average	80.2	.8	14.2	2.8
		.8	14.3	2.8
		.8	14.3	2.8

These tests were made by the Babcock Method.

Yours very truly,
AGRICULTURE DAIRY & FOOD DEPT.
E. T. Hodge
Deputy Commissioner

ELH:BG
cc Torgueson

CRISS-CROSS WORKING of the VANE CHURN

HELPS YOU GET MORE BUTTER FROM EVERY POUND OF CREAM

EVERYBODY in the butter industry knows that legal butter must contain 80% butterfat. But, according to reliable sources, the national average is over 80.7%. On the basis then, of our annual production of 1,750,000,000 pounds, we are giving away 12,250,000 pounds of butterfat or 15,190,000 pounds of butter each year. *Startling, isn't it?*

Think what this would mean to the butter shortage!

Let's grant the fact that all of this butterfat cannot be saved. Yes, let's even be conservative and say that only four-tenths of one per cent would be saved. Even that will amount to the staggering sum of 7,000,000 pounds of butterfat or 8,680,000 pounds of butter annually.

The composition record above proves that this kind of a saving is possible with a Vane Churn. There are hundreds of other Vane Churn users who are accomplishing these same results.

Yes, it is the Criss-Cross Working Principle of the Vane Churn that makes this possible. You can make more butter from the same amount of butterfat.

GENERAL DAIRY EQUIPMENT CO. MINNEAPOLIS 14, MINNESOTA

To their GOOD HEALTH!

IT'S going to take healthy soldiers, healthy war workers, healthy families and healthy communities to win this war. That's the big reason why today it's more important than ever before to maintain highest standards in the purity of the milk they drink, despite the growing problems of changing conditions and changing personnel.

And SEAL-KAPS help to maintain high standards. For SEAL-KAPS not only tightly cover the pouring lip of the bottle from dairy to delivery but they *re*-cover the bottle after every use. Thus the milk is constantly guarded against the danger of contamination and pure milk is kept pure to the very last drop.

AMERICAN SEAL-KAP CORPORATION
11-05 44th Drive, Long Island City, N. Y.
SEAL-KAP COVERS THE DANGER ZONE

Looking Forward

Wartime achievements in science are developing an era of progress which challenges the imagination.

All that Spencer is doing now—producing microscopes, periscopes, telescopes, aircraft and anti-aircraft gunsights, prism binoculars, azimuth instruments for directing artillery fire, tank sights, telescopic alidades for navigation, projectors for instruction—will reap peacetime rewards in advanced knowledge, better manufacturing techniques, finer instruments.

At the war's end, Spencer will be ready to serve scientific optical needs on a far broader scale than ever before.

Spencer LENS COMPANY
BUFFALO, NEW YORK
SCIENTIFIC INSTRUMENT DIVISION OF
AMERICAN OPTICAL COMPANY

Your advertisement is being read in every State and in 25 Foreign Countries

HEY, SARGE! LISTEN TO THIS . . .

WE KNOW what you're up against, trying to issue uniforms to a bunch of rookies! You don't mind the size 38 who wears size 9 shoes. But that big 6 ft. 7 in. bruiser who weighs 340—man, oh, man! Or the squirt who wears a 5½ shoe—they drive a guy to distraction!

And in the salt business we've got to "fit" the salt to the job. Butter makers don't want large, slow dissolving crystals in Butter Salt. We remove the "big ones" so completely, you won't find even a trace on a 28-mesh Tylor screen. But they don't want fine dust, either, to cause pasting in the churn. Diamond Crystal Butter Salt contains only 3% of particles small enough to pass through a 65-mesh screen!

Yes, it's just as exacting for a salt producer to remove over-sized and under-sized salt crystals . . . as for the "top kick" to take care of his over-sized and under-sized rookies. If we know the average "top kick," however, he gets results and so do we at Diamond Crystal. You can be sure of clean screening when you specify Diamond Crystal. Take your choice of grade or grain—it's tops by actual tests!

NEED HELP? HERE IT IS!

If *you* have a salt problem, let our Technical Director help! Just address him at Diamond Crystal, Dept. H-2, St. Clair, Michigan.

DIAMOND CRYSTAL *ALBERGER PROCESS* SALT

Your advertisement is being read in every State and in 25 Foreign Countries

HIGH TEMPERATURE SOAKING DOES IT

EVEN THE DIRTIEST BOTTLES COME OUT CLEAN AND THERE'S MIGHTY LITTLE BREAKAGE

MODEL E WASHER

To get dirty milk bottles (yes, even the dirtiest ones) really clean and sparkling demands uniform high temperatures and controlled high caustic concentrations in the soak tank. But doesn't this run up bottle breakage and the amount of washing powder used? Not in a Cherry-Burrell MODEL E WASHER. In this washer, fourteen bottle treatments permit soak tank temperatures as high as 180° with perfect safety — bottle breakage is negligible — and, what's more, *less* water and *less* washing powder are used!

BUY ANOTHER BOND

These streamlined washers handle all size bottles, from ½-pint to 2-quart, without adjustment, are exceptionally sturdy and easy to operate, are fully enclosed yet instantly accessible. Upon proper priorities, we can still furnish a very limited number in 6, 8, 10 and 12-wide sizes — capacities from 35 to 110 bottles a minute. Perhaps you are eligible for a MODEL E. If so, you are lucky — it will save you time, labor, supplies, bottles and money. Talk it over soon with your Cherry-Burrell representative or write to us for complete information — TODAY!

BUY TWO MORE BONDS

CHERRY-BURRELL CORPORATION

427 WEST RANDOLPH STREET, CHICAGO 6, ILLINOIS

★ ★ ★

FACTORIES, WAREHOUSES BRANCHES, OFFICES OR DISTRIBUTORS AT YOUR SERVICE IN 55 CITIES

Your advertisement is being read in every State and in 25 Foreign Countries

PRODUCTION TESTING

A Going Program Among Jersey Breeders

More than 100,000 Jerseys have been semi-officially tested in Register of Merit and in Herd Improvement Registry

68,012 Records completed in Register of Merit, average 8,637 lbs. milk, 5.37%, 463 lbs. fat.

35,272 Records completed in Herd Improvement Registry, average, 7,133 lbs. milk, 5.30%, 378 lbs. butterfat.

This fundamentally is the reason why Jerseys can have Selective Registration

The American Jersey Cattle Club

324 West 23rd Street

New York 11, New York

Your advertisement is being read in every State and in 25 Foreign Countries

JOURNAL OF DAIRY SCIENCE

VOLUME XXVII FEBRUARY, 1944 NUMBER 2

ACTION OF BUTTER CULTURES IN BUTTER: A REVIEW*

F. J. BABEL AND B. W. HAMMER

Iowa Agricultural Experiment Station, Ames, Iowa

CONTENTS

Introduction 80
Methods of using butter cultures in the manufacture of butter 80
Salted butter 80
Adding butter culture to pasteurized cream and holding the mixture cold for some hours 81
Adding butter culture to pasteurized cream and ripening somewhat 82
Adding butter culture to pasteurized cream at churning 84
Adding butter culture directly to butter 86
Unsalted butter 87
Use of special cultures in butter manufacture 88
Survival and growth of butter culture organisms in butter 90
Salted butter 90
Unsalted butter 92
Sources of natural butter flavor 93
Effect of butter culture on flavor of butter 94
Effect of butter culture on keeping qualities of butter 95
On general score 95
In preventing certain bacterial defects 99
Relationship of specific chemical compounds to desirable flavor of butter 101
Volatile acids 102
Acetylmethylcarbinol and diacetyl 103
Homologs of diacetyl 105
Miscellaneous 105
Factors influencing acetylmethylcarbinol and diacetyl contents of butter ... 106
Influence of strain of butter culture 106
Influence of acidity of cream 107
Influence of temperature of ripening 110
Influence of fat content of cream ... 112
Influence of addition of citric acid to cream 113
Influence of aeration of cream 114
Influence of neutralization and pasteurization of cream 116
Influence of certain metallic salts 117
Influence of churning 117
Influence of washing butter with water 117
Partition of acetylmethylcarbinol and diacetyl during churning 118
Comparative amounts of acetylmethylcarbinol and diacetyl in cream and butter 118
Comparative amounts of acetylmethylcarbinol and diacetyl in cream and buttermilk 120
Comparative amounts of acetylmethylcarbinol and diacetyl in butter and buttermilk 121
Partition of acetylmethylcarbinol and diacetyl between aqueous and fatty constituents of cream and butter 122
Influence of reworking butter 125
Influence of storage of butter 125
Salted butter 125
Unsalted butter 128
Action of acetylmethylcarbinol and diacetyl on butter 129
Chemical considerations 130
Microbiological considerations 132
Amounts of acetylmethylcarbinol and diacetyl in various dairy products 132
Milk 133
Skimmilk 133
Cream 133
Other products 134
Butter 134
Butter culture distillate as a source of flavor for butter 136
Detection of added diacetyl in butter ... 137

* Journal Paper No. J–1154 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project 127.

Copyrighted, 1944, by the AMERICAN DAIRY SCIENCE ASSOCIATION.

INTRODUCTION

The butter industry early recognized that the type of souring in cream largely determines the flavor of butter freshly prepared from it, and attempts were made to control the souring of raw cream intended for churning by addition of clean-flavored sour milk or cream, buttermilk from a churning of good butter or some similar material. Although pasteurization of cream for butter originally was intended primarily to improve the keeping qualities of the butter, it also permitted better control of the fermentation of the cream through use of selected cultures of bacteria in a medium relatively free of other micro-organisms. Pasteurization of cream and flavor development through use of selected cultures of bacteria increased simultaneously in the butter industry during the final decade of the last century, each tending to complement the other under the general system of butter manufacture then followed. With the recognition of the relationship of high churning acidities in cream to chemical deterioration in butter, use of low acid cream, either sweet or neutralized, and changes in the methods of employing butter cultures were indicated and soon were widely followed.

The present review deals with the general action of butter cultures in butter. It supplements a review (43) dealing with the bacteriology of butter cultures, including the chemical changes brought about in milk through activity of the normal butter culture species.

METHODS OF USING BUTTER CULTURES IN THE MANUFACTURE OF BUTTER

When butter cultures first came into general use, the common practice was to ripen the cream, either raw or pasteurized, to a comparatively high acidity and then cool and churn. On holding, butter made in this manner sometimes developed serious flavor defects because of the influence of acid on chemical changes in it and, accordingly, methods of employing cultures were developed which involved churning the cream at a relatively low acidity.

The method of using cultures in a plant often is determined largely by the type of butter desired, as well as by the plant equipment and plan of operation. Salted butter for immediate consumption can be churned at a higher acidity, so that it will have more flavor, than butter to be stored for considerable periods during which serious chemical deterioration may occur. With unsalted butter, a high churning acidity ordinarily is employed because in the absence of added salt a high acidity is relatively unimportant as a cause of deterioration; moreover, the flavor desired in unsalted butter intended for table use is best obtained through a high churning acidity.

Salted Butter

At present the more common methods of using butter cultures in the making of salted butter are:[1]

[1] A report involving a comparison of several methods of using butter culture is reviewed under the method which gave the best results.

Adding butter culture to pasteurized cream and holding the mixture cold for some hours. An important method of using butter culture is to add 2 to 8 or more per cent to pasteurized and cooled cream, hold the mixture cold for some hours, preferably overnight, and churn. While the added culture raises the acidity of the cream somewhat, there is no significant increase in acid during the holding because the low temperature largely prevents the action of the culture organisms.

Wilster (138) noted the most improvement in the flavor of butter when the cream, with culture added, was held 16 hours below 40° F.,[2] or when the cream was ripened to 0.28 per cent acid (0.42% in the serum) and held overnight below 40° F. With these methods the average scores of the butter were increased nearly 1 point compared to butter made without culture or to butter made with culture added to the cream immediately before churning.

In 28 comparisons, Fabricius and Hammer (33) found addition of 8 per cent culture to sweet or slightly sour cream 16 hours before churning gave butter significantly more often high in score than addition just before churning; this was the case with both the fresh and the stored butter. In 11 trials in which acetylmethylcarbinol plus diacetyl[3] determinations were made on the cream at churning, addition of culture 16 hours before churning commonly resulted in higher values than addition at churning. This was not the case in two of the trials and with these the butter made by adding culture 16 hours before churning scored the lower. In another series of experiments, no culture was added to one lot of cooled sweet cream and to other lots 8 per cent was added at various periods before churning, the periods used in the eight trials including 3, 6, 16, 40, 64, 88 and 112 hours. Butter made with culture consistently scored higher than butter made without culture, both before and after storage. Holding periods greater than 16 hours were of no advantage. Amc + ac_2 contents of the cream at churning were sometimes increased and sometimes decreased by holding for the various periods with culture added.

Wiley *et al.* (137) found that addition of 0.5 per cent culture to pasteurized and chilled sweet cream, with holding overnight at a low temperature before churning, improved the flavor of butter. No acid developed in the cream under these conditions, although the resultant butter had a somewhat "brighter" flavor than if made from sweet cream alone.

Fabricius (31) stated that when culture is added to cream at 40° F. or below and the mixture held overnight at a low temperature, considerable amc usually is oxidized to ac_2. Later (32), he noted that addition of culture to pasteurized and cooled cream several hours before churning brings about

[2] Although in the earlier review (43) temperatures are given on the Centigrade scale, the Fahrenheit scale is employed herein since so many of the results were reported on this basis.

[3] Throughout the remainder of the review acteylmethylcarbinol = amc and diacetyl = ac_2.

little change in the acidity of the cream but usually gives definite increases in the amc + ac_2 content.

Adding butter culture to pasteurized cream and ripening somewhat. Because of the danger of accelerating chemical deterioration in butter, extensive ripening of cream for salted butter is now rarely used. However, at temperatures favorable for the culture organisms, such as 70° F., slight ripening sometimes is employed, particularly with butter intended for immediate consumption. Ripening of the cream commonly is followed by cooling to a temperature which will largely prevent the action of the culture organisms, after which it is held for some hours before churning.

The Ontario Agricultural College (83) reported that an improved quality of butter for immediate consumption could be made from high acid cream by reducing the acidity, adding a good culture and ripening to a low acidity before churning.

Jackson (52) compared the scores of butter made by adding 10 per cent culture to neutralized and pasteurized cream and ripening for 30 minutes with the scores of butter made from neutralized and pasteurized cream without added culture; he found practically no difference.

White *et al.* (134) stated that ripening cream with culture, even to low acidities, improved the score of the fresh butter. In the experiments of Walts (131) butter made from neutralized and pasteurized cream with culture scored higher in all the trials than butter made without culture. The trials involved ripening the cream 2 hours at 65° F., ripening 12 hours at 65° F., ripening to 0.30 per cent acid at 65° F., ripening 1 hour at 48° to 56° F. and ripening overnight at 50° F.

According to Lucas *et al.* (65) the average score of fresh butter from cream ripened to 0.30 per cent acid was higher than that from cream to which culture was added at the time of churning, that from cream to which culture was added just before working, that from cream ripened to 0.45 per cent acid and that from sweet cream (without culture). Butter from sweet cream lacked flavor and that from cream ripened to 0.45 per cent acid had an undesirable acid flavor; after 30 days in storage, the latter had suggestions of fishy, unclean and tallowy flavors. The authors noted that even under ideal conditions it is unwise to ripen cream with culture, even moderately, if the butter is to be stored for any considerable period.

Price *et al.* (96) compared butter manufactured with and without culture. The culture was added to pasteurized and neutralized cream when it had been cooled to 70° F. After holding at this temperature until 0.28 to 0.30 per cent acid had developed, the cream was cooled to 35° to 40° F., held overnight and churned the following morning. Of 93 churnings, 59 were made with culture added to the cream and 34 were made without culture. The fresh butter made with culture ranged in score from 90.5 to 95

and averaged 93.03; butter made without culture ranged in score from 90.5 to 93.5 and averaged 91.96. After holding 1 month at 35° to 45° F., scores of butter made with culture ranged from 89 to 94 and averaged 92.07; scores of butter made without culture ranged from 89 to 94 and averaged 91.6. After holding 6 months at 0° to 10° F., scores of butter made with culture ranged from 90 to 94 and averaged 92.4;. scores of butter made without culture ranged from 89.66 to 93.5 and averaged 91.63. From the frequency distribution of the butter scores, it was noted that 93.23 per cent of the scores of the fresh butter made with culture were 92 and above as compared to 61.77 per cent for fresh butter made without culture. After holding 1 month at 35° to 45° F., 61.02 per cent of the butter made with culture scored 92 and above as compared to 49.98 per cent for butter made without culture. After holding 6 months at 0° to 10° F., 77.97 per cent of the butter made with culture scored 92 and above as compared to 44.12 per cent for butter made without culture. There was very little difference between the keeping qualities of butter made with and without culture. The scores of butter made with culture decreased more during holding 1 month and 6 months, but at the end of each holding period a considerably larger percentage of scores were above 92 than in the case of butter made without culture.

Results of Overman (86, 87) showed that butter having the highest initial score was churned from fresh cream separated from fresh, sweet whole milk. Butter made from pasteurized sweet cream after ripening with culture and partially neutralizing scored higher when fresh than butter made from the same cream after spontaneous souring and neutralizing to the same acidity.

Fabricius and Hammer (33) compared addition of 8 and of 10 per cent culture to cream after cooling with addition of 8 per cent culture to the cream at 70° F., ripening for 1 hour and then cooling. Ripening of the cream gave butter more often high in score than addition of 8 or of 10 per cent culture to the cooled cream, when the butter was fresh, after 6 weeks at 28° F. and after 6 months at 0° F. Differences in score, however, were only significant in the case of the butter held 6 weeks at 28° F. Another series of comparisons involved: (a) Addition of 8 per cent culture to pasteurized and cooled cream with holding at 28° to 36° F.; (b) addition of 8 per cent culture to pasteurized and cooled cream with holding at 41° to 52° F.; and (c) addition of 8 per cent culture to pasteurized cream at 70° F. followed by ripening for 1 hour, cooling and holding at 28° to 36° F. Butter from cream to which 8 per cent culture was added with holding at 41° to 52° F. and from ripened cream was more often higher in score than butter from cream to which 8 per cent culture was added with holding at 28° to 36° F.; however, in no case was the difference significant.

Crossley (21) stated that when growth of the culture organisms is allowed to continue in cream after adding culture, production of ac_2 is in-

creased and full-flavored butter is obtained. The acid developed during ripening, however, has an adverse effect on the keeping qualities of the butter.

Langton (59) noted that cream must be ripened in order to obtain full-flavored butter. *Streptococcus lactis* or *Streptococcus cremoris* plus flavor organisms were considered essential. Various strains of lactic acid streptococci brought about appreciable differences in the flavor of butter.

According to Fabricius (31) when culture is added to cream at 70° F. and the mixture held 2 to 3 hours before cooling, the ac_2 content often decreases and the cream must then develop about 0.40 per cent acid before appreciable amounts of ac_2 are produced.

Adding butter culture to pasteurized cream at churning. Butter culture sometimes is added to pasteurized cream at the time of churning. From the standpoint of flavor of the butter, both experimental results and commercial experience indicate that this is less desirable than holding a mixture of cream and culture cold for some hours before churning, but it may be necessary with limited vat space. It also avoids risking an increase in the acidity of the cream-culture mixture when the cooling and holding equipment are inadequate.

Dean (28) noted that the flavor of butter made from sweet cream with culture added at churning was better than that of butter made from sweet cream alone. Sweet cream churned with 27.5 per cent added culture gave better butter than ripened cream when the butter was fresh; it also held its flavor better during storage. Rogers and Gray (104) found that butter made from pasteurized cream with culture added retained its fresh flavor better than ripened cream butter.

Mortensen (80) compared butter made from sweet cream and from sweet cream to which culture was added before churning. Immediately after the butter reached the market, that made from sweet cream scored higher in 8 of 32 trials, that made from sweet cream with culture added scored higher in 16 trials and the scores were the same in 8 trials. After 2 months in cold storage (0° F.), the sweet cream butter had the higher score in 10 of 35 trials, that made from sweet cream with culture added had the higher score in 20 trials and the scores were the same in 5 trials. After 9 months in cold storage, the sweet cream butter had the higher score in 11 of 22 trials, that made from sweet cream with culture added scored higher in 8 trials and the scores were the same in 3 trials. In another experiment, ripened cream butter was compared with butter made from sweet cream to which culture was added without ripening. Immediately after the butter reached the market, the ripened cream product had the higher score in 23 of 32 trials, that made from sweet cream with culture added scored higher in 6 trials, while the scores were the same in 3 trials. After 2 months in cold storage,

the ripened cream butter had the higher score in 10 of 35 trials, that made from sweet cream with culture added scored higher in 20 trials and the scores were the same in 5 trials. After 9 months in cold storage, the ripened cream butter had the higher score in 6 of 23 trials, that made from sweet cream with culture added scored higher in 16 trials and the scores were the same in 1 trial.

According to Grimes (37) butter made from sweet cream to which culture had been added without ripening had a higher initial score than butter made from sweet cream.

Lucas *et al.* (65) found that, after 30 days at 0° F., butter made from cream having culture added at churning scored highest, butter made with culture added just before working was second, butter made from cream ripened to 0.30 per cent acid was third, butter made from sweet cream (without culture) was fourth and butter made from cream ripened to 0.45 per cent acid was fifth. The butter made from cream ripened to 0.45 per cent acid declined in average score 1.7 points during the storage while that made from cream having culture added at churning decreased in average score 0.7 of a point. After 3 months in storage the average score of the butter made with addition of culture to the cream at churning was still highest; the butter was excellent and the average score had decreased 1.1 points. Of almost equal quality was the butter having culture added just before working, the average score having decreased only about 0.5 of a point. The sweet cream butter was third and the ripened cream butter was last.

Parsons (89) made butter from split vats of cream, with and without culture. The average scores of the fresh butter showed that culture improved the product. In the individual comparisons butter made with culture scored 0.20, 0.22, 0.27, 0.32 and 0.50 of a point higher than that made without culture. Butter made with culture appeared to be slightly more uniform and had a more pleasing aroma than butter made without culture. A wider difference in score in favor of butter made with culture was found with cream graded to make a 90 to 91 score product than with cream graded to make an 89 score product.

A comparison, by Fabricius and Hammer (33), of addition of culture to pasteurized and cooled, neutralized sour cream at churning and 16 hours before churning indicated that addition at churning consistently gave butter lower in score.

Crossley (21) found it desirable to make butter from sweet cream plus culture, rather than from ripened cream, in order to insure satisfactory keeping qualities during lengthy cold storage. When this procedure is followed, reliance is placed entirely on the flavor of the culture and well-ripened culture is of paramount importance.

Davies (23, 27) stated that the most promising method of using culture consists of employing small quantities of special cultures which are allowed

to produce large amounts of ac_2; the culture is added to the cooled cream and the mixture churned without delay.

According to Hunziker (48; p. 363) some buttermakers follow the practice of adding culture to the cream just before churning in order to avoid excessive acidities through cream ripening. Use of 5 to 7 per cent culture usually raises the cream acidity approximately 0.03 to 0.04 per cent. The flavor and aroma of the resulting butter depend solely on the degree to which they are present in the culture. Hunziker concluded that the effect of culture on the butter is slight, and it is questionable whether the practice is economically justifiable.

Jacobsen and Evans (53) obtained a slightly higher general level of scores on butter made with culture than on butter made without culture, when the butter was scored fresh.

Adding butter culture directly to butter. When cream with added culture is churned, a portion of the flavor constituents in the culture is lost in the buttermilk. Because of this undesirable partition, there have been various suggestions that culture be worked into the butter. With normal churning procedures the amount of culture that can be added in this way is limited, if the usual composition standards are to be met; with special churning procedures the amount can be increased. Various butter experts originally predicted that working culture into butter would have an undesirable effect on the keeping qualities; in general, this has not been the case but with an unsatisfactory culture there may be dangers along this line.

The Minnesota Agricultural Experiment Station (74) reported that working a small amount of culture into butter at the churn produced butter with a better flavor than that made from ripened cream. Lucas *et al.* (65) concluded that working culture into butter had practically no effect on the curd content or on deterioration. Bouska (10) suggested that the cost of culture can be reduced by working it into the butter.

Hunziker (48; p. 364) stated that working the desired aroma substance directly into butter eliminates much of the added operating expense, avoids the quality-jeopardizing effect of high churning acidities and prevents waste of the flavor principle. He suggested that instead of using water to wet the salt and bring the moisture content to the desired point, several gallons of culture be added to the butter with the salt. Culture so used assists somewhat in freshening the flavor of butter made from unripened cream. It does not increase the acidity of such butter sufficiently to augment the danger of deterioration due to chemical reactions. The keeping qualities of butter so treated are similar to those of butter made from unripened cream without addition of culture. The resulting improvement in flavor, however, is slight; the butter still lacks the full flavor and aroma of ripened cream butter.

Unsalted Butter

With unsalted butter intended for table use, a high flavor is very desirable and this necessitates high ripening of the cream, whether it is sweet or neutralized sour cream. Frequently the cream is ripened to 0.45 per cent acid or more, values which often would be disastrous with salted butter because of the greatly accelerating effect of the acid-salt combination on chemical changes in butter. In other cases much lower acidities are employed. Some plants use lower ripening acidities in summer than in winter because during the warmer weather there is more opportunity for development of the culture organisms in the butter itself and this may lead to their overdevelopment, with resulting destruction of flavor constituents (43; p. 115).

High ripening of cream for unsalted butter commonly is carried out at relatively low temperatures, with the temperature often changing during the ripening period as the cream warms or cools from the air of the room. Frequently the temperature of the cream is adjusted to a point which will give the desired acidity during the normal holding period, with the usual inoculation and usual room temperature; in general, the point to which it is reduced is lower in summer than in winter and there may be a shift from day to day as the acidities developed are too high or too low.

Experiments conducted by Jackson (52) indicated that when cream was inoculated with 10 per cent culture, after being neutralized and pasteurized, and ripened 30 minutes at 70° F., the resulting unsalted butter was lower in score than that made from neutralized and pasteurized cream without added culture.

Hunziker (48; p. 363) stated that, with unsalted butter, it is desirable to ripen the cream to a full flavor and aroma. Excellent results are obtained by low-temperature ripening, such as 50° to 55° F., overnight. With this method the cream usually shows a pronounced flavor and aroma the following morning and an acidity of about 0.35 to 0.45 per cent. With ripening at higher temperatures (60° to 70° F.), the aroma character tends to be somewhat more pronounced and the acidity somewhat higher (0.5 to 0.65%).

Hedrick and Hammer (45) studied the effect of ripening cream to various acidities on its ac_2 and amc contents. Results of ten semi-commercial trials on cream (seven on sweet cream, three on neutralized sour cream) ripened to various acidities at temperatures from 50° to 55° F. showed an increase in ac_2 with an increase in acidity in nine trials (90%) and in amc in seven trials (70%). Some of the increases were small. Ac_2 decreased in one trial (10%), the drop being from 1.88 to 1.49 ppm. with a change in acidity from 0.37 to 0.49 per cent, and amc in three trials (30%).

With unsalted butter that is being stored for reconstitution into cream, ice cream mix, etc., small amounts of culture sometimes have been used, the idea being to improve the keeping qualities of the product. The culture has

been added at churning, or some hours before churning when the cream temperature is low, a significant increase in acid being carefully avoided.

USE OF SPECIAL CULTURES IN BUTTER MANUFACTURE

Special cultures, instead of the usual butter cultures, occasionally have been employed in butter manufacture, mainly on an experimental basis. They include pure cultures of the normal butter culture organisms and of various closely related species, in some instances the pure cultures being given special treatments intended to intensify flavor development in them, and also butter cultures grown under special conditions. Certain of the special cultures eventually may prove of value in commercial butter manufacture.

Hammer (38) found that addition of *Streptococcus citrovorus* and sterile lactic or citric acid to pasteurized cream resulted in a significant increase in score of the resulting butter; the increase over the control was greater with 0.2 per cent citric acid than with 0.1 per cent. Without added acid the organism gave a slight increase in score. Similar results were obtained with *Streptococcus paracitrovorus*. *S. lactis* gave a better flavor and aroma with sterile citric acid added to the cream than without the acid, but the flavor and aroma of the resulting butter were not as good as those produced by *S. citrovorus* and added citric acid. Addition of sterile citric acid without any inoculation gave a higher score than when *S. lactis* was used without added acid. Later, Hammer (39) noted that considerable flavor and aroma could be produced in butter by growing one of the flavor organisms (*S. citrovorus* or *S. paracitrovorus*) alone in cream that had been carefully pasteurized. A small amount of citric acid added to the cream before inoculation resulted in excellent flavor in the butter.

Bogdanow (8) made butter with *S. lactis*, with *S. cremoris* and with flavor organisms. The best flavor was obtained with *S. lactis*; however, the butter showed the least storage capacity. Butter made with flavor organisms alone had no flavor when fresh and scored lowest.

In the experiments of Maddock (66), butter of exceptionally fine flavor was produced in laboratory tests by use of pure cultures of *S. paracitrovorus*. However, under practical conditions the uncertainties were too great to warrant its general use, the organism being unable to compete successfully with the contaminants encountered in practice.

Fabricius and Hammer (33) compared the use of no culture, regular butter culture and modified butter culture which was prepared as follows: Whole milk was heated to 180° F. for 1 hour, cooled to 70° F. and inoculated with a pure culture of *S. paracitrovorus* in sterile milk. After incubating 24 hours at 70° F., 0.30 per cent sulfuric acid and 0.15 per cent citric acid were added. The culture was incubated another 24 hours at 70° F. and then cooled to 40° F. With sweet cream, modified culture gave butter sig-

nificantly higher in score than regular culture when the butter was fresh and also after holding a few weeks at 28° F. Regular culture gave butter significantly higher in score than no culture when the butter was fresh; after holding at 28° F. the difference was not significant, although the higher score was more often obtained with the culture. Modified culture gave butter significantly higher in score than no culture when the butter was fresh and also after holding at 28° F. Trials involving sour cream showed identical results. Comparisons also were made using no culture, regular culture and regular culture neutralized to 0.30 per cent acid. With sweet cream, regular culture and regular culture neutralized gave butter that did not differ significantly in score when fresh or after cold storage; after holding at 28° F. the butter made with neutralized culture was significantly higher in score. Butter made with regular culture was significantly higher in score than that made without culture when fresh, whereas after holding at 28° F. or after cold storage the difference was not significant. Butter made with regular culture neutralized was significantly higher in score than that made with no culture when fresh and after holding at 28° F., but after cold storage the difference was not significant. Trials involving comparisons of no culture, modified culture and modified culture neutralized were made with sweet cream. Butter made with modified culture was more often higher in score than that made with modified culture neutralized, both after holding at 28° F. and after cold storage; when scored fresh the numbers of higher scores were equal. Differences were significant only with the cold storage butter. Modified culture and also modified culture neutralized gave butter significantly higher in score than no culture in all cases. However, the difference was significant only in case of the fresh butter. Another series of trials involved adding regular butter culture and pasteurized butter culture to sweet cream. The pasteurized culture was made by heating ripened culture to 145° F. for 30 minutes and filtering off the curd. The serum was cooled to 40° F. and used in cream. Butter made with regular culture was more often higher in score than butter made with pasteurized culture when fresh, after holding at 28° F. and after cold storage; differences were significant when the butter was fresh and after holding at 28° F.

Matuszewski *et al.* (69) made laboratory churnings using 5 per cent of a culture of *Streptococcus diacetilactis* to ripen the cream to about 0.5 per cent acid at 68° F. Flavor of the butter was good, but at times was too high, especially after 3 to 5 days at 50° to 59° F. When *S. diacetilactis* was used in combination with *S. cremoris* and *S. citrovorus*, there was an improvement in the butter compared to butter made with regular culture.

Comparisons were made by Brewer *et al.* (11, 12) to determine the quality of butter produced by use of aerated-pressure butter culture. These usually were prepared by adding 2 per cent active culture to pasteurized skimmilk, although in some cases whole milk was used to prevent excessive

foaming during aeration. After inoculation the milk was divided, one portion being ripened with aeration under 60 pounds pressure, while the other was ripened as usual to serve as a control. The ripened cultures were cooled and then added to divided lots of pasteurized sweet cream. After appropriate holding, the mixtures were churned. Results showed that aerated-pressure culture gave butter scoring 0.15 to 1 point higher than that made with regular culture; with the aerated-pressure culture the flavor was higher with both sweet and sour cream butter. In trials in which 5 per cent regular culture and only 2 per cent aerated-pressure culture were added to cream, the butter made with the aerated-pressure culture regularly had the higher flavor.

Khubchandani (55) found that use of *S. citrovorus* cultures, treated with citric acid or sodium citrate 24 hours prior to inoculation into cream, gave better flavor and aroma in butter than use of untreated cultures. The keeping qualities of such butter were not affected by the citric acid or sodium citrate.

Davies (27) attempted to produce butter of average flavor and low acidity by use of pure cultures of *Betacoccus cremoris* and *S. paracitrovorus*. The cultures were employed successfully in the laboratory but became contaminated in plants and finally were essentially regular butter cultures.

SURVIVAL AND GROWTH OF BUTTER CULTURE ORGANISMS IN BUTTER

Rapid growth of the butter culture organisms in milk, cream, etc., at favorable temperatures suggests that they would grow rapidly in butter. However, in the case of salted butter the added salt is an important inhibiting factor and with both salted and unsalted butter the low holding temperatures often used and the small amounts of milk solids-not-fat present also are of significance in limiting the growth. Butter culture organisms sometimes survive for extended periods in butter.

Salted Butter

Sayer *et al.* (109) found a rather regular decrease in the numbers of lactic acid bacteria in butter during storage at 41° F. The decrease was related to the concentration of salt in the butter, higher concentrations giving greater decreases. Apparently the salt content was not the only reason for the decrease in numbers.

Washburn and Dahlberg (133) reported that *S. lactis* withstood salt and low storage temperatures better than any other organism in butter. It was the only species appearing regularly in all lots of butter. After 304 days in storage, every lot of salted butter contained essentially only *S. lactis*. Brown *et al.* (15) noted that lactic acid bacteria persisted in butter for as long as 275 days (in one case 426 days). According to the authors salt and low temperatures may cause such a slow rate of metabolism that relatively

little acid is produced and life of the cell is prolonged. Butter in storage showed a gradual decrease in the amount of lactose and a gradual increase in acidity.

Grimes (37) found that butter made from cream to which culture had been added, either without ripening or with ripening to various acidities, retained per ml. from 0.5 to 2.0 per cent of the culture organisms per ml. of the cream-culture mixture. The flora of the butter consisted of *S. lactis* and *S. paracitrovorus, S. lactis* making up 70 to 100 per cent of it. After 6 months at – 6° F. over 98 per cent of the bacteria had died. After cold storage, sweet cream butter often had a higher bacterial count than butter made from cream to which culture had been added. In general, it appeared that the normal flora of pasteurized cream found an acid environment unfavorable; also, utilization of lactose by *S. lactis* and *S. paracitrovorus* was thought to be a factor. The flora of the butter when taken out of cold storage consisted chiefly of *S. lactis* and acid-forming, non-coagulating species. *S. paracitrovorus* usually was present. *S. lactis* was not found in pasteurized cream butter made without culture when first manufactured but was found after cold storage.

It was noted by Parfitt (88) that salted butter made with culture showed less variation in pH during storage than butter made without culture.

In the trials of Nelson and Hammer (81) butter was churned under both laboratory and commercial conditions. With the laboratory butter, one series of trials involved butter made from cream of poor quality so that the microscopic count of the fresh control butter was high, considerable numbers of both streptococci and other organisms being present. During holding at 69.8° F. the numbers of organisms in the control butter showed comparatively little change. With three of the organisms (butter culture types) used in the cream, the butter showed fluctuations in the numbers of streptococci during the holding in both the plate and microscopic counts, but there were no definite trends evident. With the fourth culture organism used, each method of counting indicated a definite increase in the numbers of streptococci. Throughout the examinations the microscopic counts regularly were much higher than the plate counts with both the streptococci and the other organisms, and the ratio between the two was extremely variable. Another series of trials involved cream of very good quality and the microscopic and plate counts on the fresh control butter were low and did not show significant changes during the holding. With the butter made from cream inoculated with culture organisms, there was a general tendency for a decrease in the numbers of streptococci with both methods of counting, and the decrease definitely was greater on a percentage basis with the plate method than with the microscopic method. With butter made under commercial conditions, the general change in the numbers of streptococci, as shown by the microscopic counts, was a decrease that commonly was exten-

sive; with certain samples it was very regular while with others some fluctuation occurred. With several samples there was a suggestion of a small increase in the numbers of streptococci after 1 day, but the evidence was not at all convincing. Plate counts showed the same general changes in numbers of organisms. The streptococci appeared to be greatly autolyzed and the chains seemed to be shorter after 7 days than after 2 days.

Unsalted Butter

Rahn *et al.* (100) noted that all lots of butter made without salt and stored at 42.8° F. showed a very high increase in acidity, compared to lots of salted butter. When held at 21.2° F. the unsalted butter showed a slightly higher increase in acidity than the salted butter.

Nelson and Hammer (81) found a definite development of the butter culture streptococci in unsalted butter held at a favorable growth temperature. It was evident from both plate and microscopic counts on butter prepared in the laboratory, using a pure culture of *S. lactis* or one of the flavor organisms, and also on butter prepared under commercial conditions with butter culture. Growth resulted in the presence of long chains of streptococci and these were striking evidence of multiplication since they were not found in the salted butter or in butter (salted or unsalted) made without addition of organisms to the pasteurized cream. In the unsalted butter there also was extensive growth of organisms other than streptococci, as shown by both plate and microscopic counts.

Hammer (42) noted that in unsalted butter made with butter culture and held at 69.8° F., acidity of the serum increased more rapidly and reached higher final values in underworked butter than in thoroughly worked butter; the relationship was evident with either a small or a large inoculation of the cream.

Effect of reworking on changes in the pH of serum obtained from unsalted butter made with culture was investigated by Long and Hammer (64); pH determinations were made at once, at reworking and at different periods following the reworking. In the laboratory studies, some trials involved underworked and moderately worked butter, while others involved moderately worked and thoroughly worked butter. The more the control butter was worked, the slower were the decreases in pH of the serum. Reworking regularly resulted in a more rapid decrease in pH of the serum, and this was evident at each examination. In two trials on underworked and moderately worked butter the differences were considerable, while in one trial, also on underworked and moderately worked butter, the differences were less extensive. In one trial the control portion of the thoroughly worked butter showed relatively little decrease in pH of the serum throughout the holding period, while the reworked portion showed considerable change; the final pH value of the serum of the reworked portion was rela-

tively high compared to those of underworked and moderately worked butter similarly treated, suggesting that thorough working of the original butter may tend to counteract the effect of reworking. Butter churned under commercial conditions with a relatively large amount of culture in the cream showed the same general decrease in pH of the serum as butter made under laboratory conditions.

SOURCES OF NATURAL BUTTER FLAVOR

With butter made from raw cream allowed to sour spontaneously, the flavor is largely determined by the changes that go on during the holding of the cream, particularly those due to micro-organisms. When the fermentation is favorable the flavor of the butter is desirable, and when it is objectionable the flavor of the butter is undesirable. A desirable flavor is due to action of certain types of bacteria which include the species normally present in butter cultures and perhaps others as well. Actually, butter cultures were developed with the idea of duplicating the flavor of the best of the butter made from naturally soured cream.

Butter made without culture from cream in which there has been no appreciable bacterial action has a flavor which is due to the milk constituents, particularly the fat, plus flavoring materials absorbed or added during production and handling of the milk, cream or butter. The feed, season, stage of lactation of the cows and various other factors may influence the composition of the milk and the flavor of butter made from it. Even slight bacterial action in the milk or cream may modify the flavor. For many consumers, proper use of butter culture definitely improves the flavor.

Conn (18) noted that the flavor of butter is not due to butterfat but to certain volatile products present in small quantities. The odors produced by various bacteria plainly indicated that volatile substances were being produced during the ripening of the cream as a result of the fermentation. It was impossible to notice the odors produced by the growth of various species of bacteria without being convinced that they had something to do with the superior flavor of butter made from ripened cream.

Ruehle (108) indicated that the flavor of butter is dependent on the type of fermentation which occurs in the cream previous to churning. Hammer (41) stated that the desirable flavor and aroma of butter come from two main sources. With butter made from cream in which there has been no development of micro-organisms, the desirable flavor comes from the milk constituents present, particularly the fat, while with butter in whose manufacture culture has been used, the desirable flavor comes from the milk constituents and also from the products formed by the culture organisms. Ruehe (106) suggested that the flavor and aroma of butter are the grand total of the natural flavor of fat as it is normally produced by the cow, plus the flavors of the ingredients added during manufacture, plus the flavors

and odors absorbed during handling and storage, plus flavors caused by activity of micro-organisms, plus flavors which result from chemical changes in the product. Neuberger (82) noted that the amount of butter aroma depends on the season of the year and feed of the cows.

Another general comment (103) stated that the flavor of butter is due primarily to the natural flavor of the cream from which it is churned. Butter made from cream in which there has been no bacterial fermentation has a characteristic flavor, agreeable but mild. The nature of the flavor is dependent to some extent on the feed and may be affected unfavorably by ingestion of highly-flavored feeds of various kinds. Fat is a solvent for many flavor and aroma-producing substances and the odors and flavors to which milk or cream may be exposed, as well as those formed in the serum by bacteria, yeasts or molds, may be carried into the butter.

Results obtained by Sherwood and Hammer (113) suggested that seasonal variations in flavor and aroma of butter are not related to differences in the citric acid content of the milk or cream. Experiments conducted by Steuart (118) indicated that the composition of the fat is an important factor in the production of aroma.

EFFECT OF BUTTER CULTURE ON FLAVOR OF BUTTER

Following the introduction of butter cultures, their use spread rapidly, which definitely indicates they are of value in improving the flavor of butter. Many plants continue to use culture even when there are serious difficulties in the procurement of milk for it. In others, use of culture has been discontinued, the idea often being that culture increases the cost of butter manufacture and that the flavor improvement obtained is not reflected in a price increase.

Over the years there have been various reports covering the effect of culture on the flavor of butter. A number of these are reviewed in the consideration of the methods of using butter cultures in the manufacture of butter but there are others of significance.

Bouska (10) indicated that if butter made without culture lacks aroma, it is handicapped in maintaining its advantage as a spread for bread in competition with substitutes.

Wilster (138) stated that many experienced creamerymen and butter dealers believe the use of fine-flavored culture in first-grade, neutralized sour cream and in sweet cream results in butter which scores higher in flavor than that made without culture. When butter is sent to a scoring contest, culture is used with the majority of the churnings. The average score of butter made with culture usually is higher than that of butter made without culture. In a cold storage contest of the National Creamery Buttermakers' Association at St. Paul, Minnesota, in 1924, culture butter averaged 0.93 of a point higher in score when fresh than butter made without culture. In a

similar contest at Cleveland, Ohio, in 1928, culture butter averaged 0.65 of a point higher in score when fresh than butter made without culture. At the Pacific International Dairy Products Show at Portland, Oregon, in 1931, butter made with culture averaged 1.40 points higher in score than butter made without culture. At the Oregon Butter and Ice Cream Makers' Annual Convention at Corvallis, Oregon, in 1932, butter made with culture averaged 0.67 of a point higher in score than butter made without culture. At the Pacific International Dairy Products Show at Portland, Oregon, in 1935, butter made with culture averaged 0.80 of a point higher in score than butter made without culture.

Fabricius (31) listed the arguments in favor of use of butter culture as follows: (a) Overcomes heated flavor, (b) decreases lime and burnt neutralizer flavor, (c) helps to overcome some feed defects, (d) may help to overcome tallowy flavors and (e) in unsalted butter its use decreases danger of cheesy, putrid and surface taint flavors. The arguments against the use of butter culture were listed as follows: (a) Cost, (b) improper use may cause oily and fishy flavors, (c) accentuates some feed flavors, (d) good culture is difficult to make and (e) price differential on the market does not justify its use.

Hoecker and Hammer (47) found that butter made with butter culture, *S. citrovorus, S. paracitrovorus* or *Streptococcus citrophilus,* usually contained relatively large amounts of ac_2 and amc and commonly placed high in a series of churnings. However, butter containing exceptionally large amounts of ac_2 and amc sometimes placed low and was criticized as being coarse, sour, oily or containing some other objectionable flavor. Butter made with *S. diacetilactis* usually contained large amounts of ac_2 and amc but often was placed low because of off flavors present. Butter made without culture or with *Streptococcus aromaticus* contained only small amounts of ac_2 and amc and usually placed low.

EFFECT OF BUTTER CULTURE ON KEEPING QUALITIES OF BUTTER

Consideration of the effect of butter culture on the keeping qualities of butter involves two distinct relationships. The first is the effect on the general score and concerns largely chemical changes in the butter, although with some lots of butter bacterial changes also are of significance, while the second is the effect in preventing certain bacterial defects of butter.

On General Score

Use of culture in butter manufacture gives the product more flavor but, because of the influence of serum acidity on chemical changes, it may result in serious deterioration. In salted butter a high flavor due to culture and good keeping qualities over long periods are more or less incompatible. Present methods of using culture are intended to give as much flavor im-

provement as possible without seriously affecting keeping qualities, the period the butter is to be held being considered.

It should be recognized that serum acidity is only one of the factors involved in chemical deterioration in butter and that some of the changes attributed to acid may have been greatly influenced by some other factor, such as contamination with copper. A serum acidity which does not result in significant deterioration when the copper content of the butter is low may be very serious when the copper content is high (50).

The early studies of Rogers and Gray (104) indicated that butter made from pasteurized cream with culture added does not have as good keeping qualities as that made without culture. A report from the Ontario Agricultural College (83) suggested that butter of improved quality may be made from high acid cream by neutralization and addition of good culture. A low churning acidity was more favorable to good keeping qualities in butter than a high churning acidity.

Mortensen (80) found that butter made from sweet cream with culture added scored higher after 2 months at 0° F. than butter made without culture, while after 9 months it scored lower. Butter made from sweet cream with culture added scored higher after 2 months and also after 9 months than butter made from ripened cream.

White *et al.* (134) stated that the improvement in flavor of fresh butter resulting from addition of culture to the cream usually is lost when the butter is placed in cold storage.

According to Lucas *et al.* (65) addition of a moderate amount of culture to cream before churning, or to the butter at the time of working, caused the butter to score higher after 3 months at 0° F. than if none had been used. Whether the aroma due to the culture covered the slight off flavors that normally develop in sweet cream butter during storage, or whether some inhibitory action took place when culture was used, the authors were unable to say. Ripened cream butter deteriorated more rapidly during storage than butter from sweet cream, butter from sweet cream with culture added just before churning or butter to which culture was added at the time of working. No relationship was found between use of culture and the iodine number of butterfat from the butter. The increase in acidity of cream obtained by using culture seemed to cause a slight increase in the acid number of the fat.

Results obtained by Hammer and Jensen (44) indicated no evident relationship between acidity of butter culture and quality and keeping qualities of butter made with it from sweet cream pasteurized at 145° F. for 30 minutes. When fresh, and also after storage, butter made with high acid culture and butter made with low acid culture ranked high or low approximately the same number of times. The small variations noted in the deterioration of the butter in storage seemed to be due to much or little

deterioration in one or two lots and did not regularly follow differences in acidities of the cultures. There was no evident relationship between acidity of the culture and quality and keeping qualities of butter made from neutralized sour cream pasteurized at 145° F. for 30 minutes. A somewhat greater variation was noted in the deterioration during storage than with butter made from sweet cream, and the difference was most conspicuous in the butter made with high acid culture; however, the variation did not follow the acidities of the cultures. Acidity of the culture would not be expected to have a great effect on the quality of fresh butter made by adding 10 per cent culture to pasteurized cream without ripening. With this method of manufacture, the flavor and aroma of the butter are directly influenced by the flavor and aroma of the culture, so that with cultures of good quality variations in acidities would be comparatively unimportant. Neither would large variations in the deterioration of the butter be expected to follow variations in acidities of the cultures because the great dilution of the added culture with cream of a much lower acidity results in only very small differences in the acidities of the butter serum. Butter made from neutralized sour cream showed a somewhat greater variation in score decreases during storage than butter made from sweet cream; this may have been due to differences in the quality of the cream.

Parsons (89) found little difference in keeping qualities of butter made with and without culture.

Results of Overman (86, 87) showed that the lots of butter which held their score best were churned from fresh cream separated from fresh, sweet milk; they were not salted and were salable after about 2 years in storage. Butter made from pasteurized sweet cream after ripening with culture and then partially neutralizing was correspondingly better than butter from the same lots of cream after spontaneous souring and neutralizing to the same acidity. Butter made from cream ripened with culture to 0.44 per cent acid and churned without neutralization scored higher and held its score better in storage than butter from the same lots of cream after ripening with culture to 0.61 per cent acid and churning without neutralization; the latter was correspondingly better than butter from the same lots of cream after ripening with culture to 0.82 per cent acid and churning without neutralization.

Bogdanow (8) reported that ripening cream with culture decreases the storage capacity of the butter.

Templeton (121) found that the storage qualities of butter made from cream in which culture, containing added citric acid or sodium citrate, had been used were very good. This was indicated by the fact that numerous samples which had been in storage for 6 weeks received higher scores than when fresh.

Wilster (138) noted that in the scoring contest conducted by the National Creamery Buttermakers' Association at St. Paul, Minnesota, in 1924, butter

made with culture scored 0.87 of a point higher after 4 months in storage than butter made without culture. In a similar contest at Cleveland, Ohio, in 1928, butter made with culture scored 0.61 of a point higher after 4 months in storage than butter made without culture. Wilster stated that approximately 100,000 pounds of butter are made annually in the Oregon State College creamery. Culture always is added to the cream and the cream is ripened to a serum acidity of 0.42 to 0.44 per cent. About 15,000 to 20,000 pounds of butter are stored during the summer for consumption in the fall. No objectionable flavors had developed in the butter made during the 6 years in which culture had been employed. There was no marked decrease in the score of butter made with or without culture during 1 month at 35° to 45° F. However, a decrease in score was observed when butter was stored 6 months at 0° to 10° F. The average decrease in score of the butter made without culture was 0.57 of a point. The average decreases for butter made with culture were 1.15 points when the cream was ripened, 0.74 of a point when culture was added and the cream held cold 16 hours previous to churning and 0.95 of a point when culture was added and the cream churned immediately; the average scores after 6 months, compared to that of butter made without culture, were 0.30, 0.66 and – 0.06 of a point in favor of the respective methods of using culture. In one series of churnings, butter was made from 93 lots of cream, culture being used with 59 and no culture with 34. When the butter was stored 1 month at 35° to 45° F. and 6 months at 0° to 10° F., the average scores of the butter made with culture were higher than those of the butter made without culture. Chief comments of the judges on the butter made with culture were "creamy flavor," "sweet and clean," "fine aroma" and "fine flavor"; those on the butter made without culture were "flat," "lacking character," "insipid" and "tallowy." (See also 96.)

The results of Wiley (135) indicated that the presence of culture organisms in butter favored oxidation of the fat at storage temperatures. The oxidation that occurred in the presence of culture organisms, even when the acid produced in the culture was neutralized before churning, demonstrated the presence of some fat-oxidizing system in the ripened cream. This was believed due to some product of bacterial metabolism acting as a pro-oxidant; if this is the case, the product is not amc or ac_2. Wiley considered the results may be explained by assuming that the lactic acid streptococci of the culture contain a fat-oxidizing enzyme which is most active at low pH and high salt concentration. Several mixed cultures (*S. cremoris* and betacocci) and a single strain of *S. cremoris* all increased oxidation, as compared to simple acidification of the cream.

According to Hunziker (48; p. 357) there are certain conditions of butter manufacture which permit controlled ripening without jeopardizing keeping qualities, and there are still other conditions of manufacture and distribu-

tion under which ripening to a full aroma and flavor is definitely beneficial to keeping qualities. The extent to which cream may be ripened and still have the butter reach the consumer without objectionable flavor deterioration due to chemical changes was considered to be influenced by such factors as: (a) Condition and quality of the original cream, (b) temperature at which butter is held between churn and consumer, (c) whether butter is intended for fresh consumption or for cold storage, (d) whether the cream is used for salted or unsalted butter and (e) salt concentration in salted butter. Hunziker (48; p. 353) also stated that cream ripening does not improve the chemical stability of butter and that, under average commercial conditions of manufacture, the ripening of cream to a full flavor and aroma shortens the life of salted butter. Usual flavor defects which develop with age in salted butter made from fully ripened cream were said to be oily-metallic, fishy and sometimes tallowy flavors. Salted butter made from sweet, unripened cream or from sour cream neutralized and pasteurized was said to keep better, from the standpoint of flavor deterioration due to chemical causes, than salted butter made from the same cream ripened to full flavor and aroma.

Jacobsen and Evans (53) found that butter made from first-grade cream without culture had better keeping qualities than butter made from first-grade cream with culture; the butter was scored after 3 and 5 months at 32° to 36° F.

In Preventing Certain Bacterial Defects

Growth of various bacteria responsible for development of specific defects in butter apparently often is delayed or prevented by use of butter culture in the cream. The inhibitory effect apparently is due not only to the products formed by the culture organisms but also to the presence of the organisms themselves.

Orla-Jensen (84) inoculated butter with *Pseudomonas fluorescens* and *S. lactis*. He noted that the lactic acid formed by *S. lactis* inhibited fat hydrolysis; there was little free volatile acid produced and the odor and taste of the butter were not especially objectionable.

Rogers (102) stated that during ripening of cream the lactose is partially fermented to lactic and similar acids which protect the butter from fermentation by less acid-tolerant organisms. The putrefactive bacteria, which often attack the curd of butter, usually were checked by the acid produced during cream ripening.

Results obtained by Mazé (70) indicated that micro-organisms causing deterioration in butter largely attack the casein and lactose. They were found to be retarded by lactic acid. Addition of lactic acid to butter at the rate of 0.5 to 1.0 g. per l. was suggested as a control measure. Lactic acid was not believed to completely prevent growth.

Shutt (114) reported that churning cream at an acidity of not less than 0.35 per cent was advantageous in preventing defects caused by *Ps. fluorescens* since it grows only feebly at a pH of 6.6. He was of the opinion that surface taint could be caused by *Ps. fluorescens*, but that it occurred only in sweet cream or neutralized cream butter and never in sour cream butter.

Virtanen (127) noted that water types of micro-organisms, which frequently cause cheesy and putrid defects in butter, are inhibited by the acid of sour cream butter.

Derby and Hammer (30) made butter from pasteurized cream inoculated with surface taint butter and studied the influence of salt and butter culture on production of the defect. Unsalted and low salted butter made without the culture developed surface taint in 2 days at 70° F., but medium salted butter made without the culture, unsalted butter made with the culture and salted butter made with the culture was normal after 7 days. Claydon and Hammer (17) added *Pseudomonas putrefaciens* to pasteurized cream and churned the cream with various amounts of butter culture added. None of the unsalted or salted butter made with the culture developed the putrid defect in 6 days at 70° F. but that made without the culture became markedly putrid. Five per cent culture inhibited the defect as effectively as 12 per cent. Results indicated that butter culture had an inhibiting effect on development of the putrid defect in unsalted butter when *Ps. putrefaciens* gained entrance to the butter either from cream or from wash water.

According to Knudsen (57) ripening cream with butter culture protects the resulting butter from attack by putrefactive bacteria which may get into it from water, but which cannot develop because the reaction is acid; ripening the cream with culture promotes development of certain defects, particularly in storage. Minster (75) considered the presence of a few lactic acid bacteria in unsalted butter as beneficial, since they tended to keep down growth of objectionable species.

In the trials of Hussong *et al.* (49) butter made from pasteurized cream inoculated with *Pseudomonas fragi* developed a rancid flavor somewhat less rapidly when 10 per cent butter culture was added to the cream just before churning than when the butter was made without culture.

St. von Nyiredy (119) reported that in butter made from ripened cream, coliform, proteolytic and *Ps. fluorescens* organisms showed practically no growth and decreased rapidly, while they increased markedly in sweet cream butter. The ripened cream butter had a pH of 4.6 to 4.8 and the sweet cream butter a pH of 6.4 to 6.8. It was concluded that failure of these groups to grow in ripened cream butter was due to the low pH.

Hunziker (48; p. 295) stated that efficient pasteurization of the cream, preferably followed by proper ripening with good culture, not only retards the general age deterioration which is characteristic of all unsalted butter but prevents the early appearance of the more serious flavor defects result-

ing from the presence in cream of specific flavor-damaging organisms. When unsalted butter was made from cream ripened to full flavor and aroma, bacterial deterioration was very noticeably retarded by the predominance of lactic acid bacteria and the relatively high acidity. These agencies assisted in checking the organisms which, if present, caused bacterial flavor defects in unsalted butter (48; p. 360). In comments on cheesy flavor, Hunziker (48; p. 668) noted that resistance of unsalted butter to bacterial age deterioration and to development of cheesy flavor is materially improved by ripening the cream with a good culture to a pronounced culture character and a reasonably high churning acidity (preferably about 0.35 to 0.45 per cent). It appeared that the great predominance of culture bacteria and the products of cream ripening are more or less antagonistic to the organisms responsible for cheesy flavor and definitely assist in holding them in check.

Fouts (35) studied the effect of butter culture and lactic acid on hydrolysis of fat in cream by pure cultures of lipolytic bacteria. Butter culture added to the cream definitely inhibited *Achromobacter lipolyticum*, *Alcaligenes lipolyticus* and *Ps. fluorescens*. When lactic acid was added to the cream used, all the organisms grew, even with enough acid to give a titrable acidity of 1 per cent.

Results obtained by Itzerott (51) showed that cream acidities below 0.15 per cent had little effect on the period required for development of the putrid defect in butter. Above 0.15 per cent, however, the acidity appeared to have a retarding influence. High acidities and low temperatures definitely inhibited the defect. Unsalted butter made from cream with 0.20 per cent acid and inoculated with rabbito organisms showed no evidence of the defect when held 3 weeks at 40° to 55° F. At higher temperatures (80° to 90° F.) the taint developed, even with a high acidity.

Pont (95) indicated that, within the range of safe limits from the standpoint of chemical changes in butter, high acidities aided in minimizing the putrid defect of butter.

Effect of butter culture on the ability of 52 fluorescent bacteria to produce defects in experimental butter was studied by Garrison and Hammer (36). Addition of 10 per cent culture to the cream prevented development of off flavors in unsalted butter by 16 of the cultures and in salted butter by 27 of them.

RELATIONSHIP OF SPECIFIC CHEMICAL COMPOUNDS TO DESIRABLE FLAVOR OF BUTTER

The desirable flavor produced in butter through use of butter culture is dependent on various compounds, some of which still may not have been identified. Ac_2 apparently is the most important of these but others, especially volatile acids, also are of significance. Ac_2 and volatile acid are

produced in butter culture, and also in ripening cream, primarily from citric acid by the normal flavor organisms while lactic acid is produced primarily from lactose by *S. lactis* (43; p. 84).

Volatile Acids

In 1890, Conn (19) stated that it usually is believed the flavor of butter is due to certain volatile fatty acids formed during the ripening of cream. The acids considered to be important were butyric, caproic, caprylic and capric.

Ferris *et al.* (34) determined the volatile acids in 14 lots of commercial sweet cream butter scoring 93 to 94. The values, expressed as ml. N/10 acid per 100 g., ranged from 0.2 to 0.4 by steam distillation and from 0.5 to 0.8 by direct distillation. The average score of the lots after storage 5 to 6 months at 22° F. was about 1 point lower than when they were fresh and the volatile acid values were about twice as large. After 6 to 7 months, the butter was removed from storage and kept 2 weeks at about 59° F. This caused a decided drop in score but no significant increase in volatile acid. More volatile acid was found in butter made from clean acid cream than in butter made from sweet cream.

Lind (63) determined the volatile acidity of butter by adding 150 ml. water and 15 ml. N/1 sulfuric acid to 100 g. butter and distilling with steam. From values obtained on skimmed milk, pasteurized cream and butter, the author concluded that butter dissolves a part of the volatile acid formed by the bacteria of the culture employed for acidification of the cream.

Results of Steuart (118) indicated that when full-flavored butter is distilled with steam, free butyric acid can be identified in the distillate as well as a neutral compound yielding butyric acid on saponification. Use in margarine of cultures producing butyric acid or addition to margarine of ethyl butyrate or butyric acid gave unsatisfactory results. Butyric acid itself was too fugitive, and a search was made for a parent substance which would continuously yield traces of the acid. In experiments with margarine prepared with skimmilk properly soured by lactic acid and aroma bacteria, it was found that a small addition of tri-butyrin caused the mass to be gradually pervaded by a pleasant butter aroma. Unfortunately, the flavor was masked by bitterness of the tri-butyrin. This compound was obtained from various sources and was synthesized, fractionated and refined, but in no instance was the bitterness avoided. Glycol di-butyrin produced a similar aroma in margarine but also had an inherent bad flavor. Mixed butyric tri-glycerides were then prepared and the flavor of these indicated that the butyric acid in butterfat must be present as a monobutyric tri-glyceride, with butyric acid in the beta position. The bitterness of tri-butyrin is due to butyric acid in the alpha position. Coconut oil was butyrised by heating with butyric acid at 320° F. After treatment for 2

hours the neutralized fat gave Gilmour butyric numbers equivalent to 3 per cent butter and after treatment for 36 hours the numbers were equivalent to 116 per cent. Fat also could be butyrised by heating it with tributyrin in the presence of a suitable catalyst, such as 1 per cent stannous hyroxide, at 392° to 482° F., the mixture being agitated by allowing an inert gas to bubble through it. Coconut fat containing from 5 to 15 per cent tri-butyrin was free of bitterness after 2.5 to 7 hours; while arachis oil with 25 per cent tri-butyrin required treatment for 11 hours.

Acetylmethylcarbinol and Diacetyl

van Niel *et al.* (126) studied the relationship of amc and ac_2 to the aroma of butter. Analyses on butter, using 50 g. portions, showed that lots having a typical aroma gave an amc reaction while lots lacking such an aroma did not. A series of lots that had been given definite scores on aroma showed a rather close correlation between a high aroma and the presence of amc. The authors further observed that a dilute aqueous solution of amc had an odor suggesting butter and that ac_2 also had such an odor. However, properly purified amc was odorless. The results suggested that ac_2 is responsible for the flavor of butter. When 0.0002 to 0.0004 per cent ac_2 was added to butter lacking aroma, an unmistakable aroma appeared. The authors concluded that ac_2 is either responsible for the aroma of butter or is the principal component of the aroma material.

The conclusion of Testoni and Ciusa (123) was that the presence of ac_2 in butter is accidental and that ac_2 cannot be considered one of the aromatic principles of butter.

Waser and Mohler (132) noted that even after purification, and in very dilute solution, ac_2 displayed the characteristic odor of butter. Tapernaux (120) stated that the artificial perfumes with an ac_2 base have given good results in creating the artificial aroma of butter in various fatty materials. Development of aroma in natural butter was believed due to the formation of ac_2 by fermentation. Ac_2 was not considered the only substance responsible for development of the natural aroma, but it was believed to be the fundamental base of butter aroma since small amounts possessed a very penetrating odor.

Kay (54) reported that the flavor, and particularly the aroma, of butter is due largely to the presence of ac_2. In commenting on this idea, Cox (54) stated that he had tested many samples of butter but had never found ac_2. The compound was not considered present in natural butter.

According to Hammer (40) the odor of ac_2 in high concentrations is pungent and not at all pleasing; however, in low concentrations the aroma is like the aroma of fine butter made from clean-flavored sweet cream with use of culture. Results obtained by Barnicoat (6) indicated that a fraction of a part of ac_2 per million imparts a rich aroma to butter made from un-

ripened cream. Examination of mild-flavored butter showed amc to be present.

Pien *et al.* (91) stated they had good reason to assume that ac_2 is not the cause of aroma in butter even though dilute aqueous solutions have an odor resembling butter and addition of ac_2 to butter without aroma gives an unquestionable aroma.

Virtanen and Tarnanen (129) considered ac_2 to be the substance responsible for the flavor of butter. Davies (23) reported that the compound responsible for most of the flavor of butter is the diketone, ac_2, which can be produced synthetically or biologically. Butterfat itself was believed to contribute to the flavor, as well as traces of esters, such as ethyl butyrate, caproate and lactate, the curd and the salt. According to Slatter (116) the desirable flavor and aroma of butter are due chiefly to the fat, volatile acids and ac_2.

Analyses by Pien *et al.* (92) showed that butter high in ac_2 had a very desirable flavor and aroma. However, many samples low in ac_2 also were excellent in flavor and aroma. Results indicated that constituents other than ac_2 are responsible for the desirable flavor and aroma of butter. Pien (90) noted that when ac_2 was absent in butter, the aroma of the butter was weak or there was no aroma.

Mazé (71) stated that the normal lactic acid organisms produce neither amc nor ac_2 and the presence of these compounds in butter proves the existence of an incidental and noxious fermentation. Ac_2 was believed to occur in the blood and, because of this, traces of it were thought to be carried into the milk.

Results of Davies (25, 26) indicated that the flavor and aroma of ripened cream can be closely simulated by working synthetic ac_2 into flavorless butter. However, this procedure gives a somewhat harsh flavor which may be due to the fact that the added ac_2 does not have the same opportunity to distribute itself between the fat and water phases of butter as the ac_2 slowly formed during cream ripening, or it may be due to modification of the effects of ac_2 by other flavoring compounds, such as esters and other volatile organic compounds, which are produced in traces in the cream. The author indicated it is generally agreed that the compound responsible for the aroma and flavor of butter is ac_2 and that it is formed together with somewhat larger amounts of amc.

Experiments by Makar'in (68), designed to test previous theories regarding formation of aroma in butter, indicated that the most important part is played by amc and ac_2.

Pont (94) noted that it is generally agreed the intensity of flavor and aroma of butter varies with the ac_2 content. Where full development of the butter culture in the cream was permitted, it appeared that the flavoring element existed mainly in the form of amc due to the low oxidation-reduction

potential of the lactic acid fermentation. Besides ac_2, other flavor contributants were butterfat and traces of esters like ethyl butyrate, caprate and lactate.

According to Langton (59) ac_2 is the cause of the aroma of butter. Davies (27) stated that addition of ac_2 to fats in amounts equal to those found in natural butter gives a harsh, artificial flavor. He suggested that when synthetic ac_2 is added, only about one-tenth of the amount found in butter of equal flavor should be employed. The main argument in favor of the use of synthetic preparations was that non-flavored butter intended for long keeping could be made from sweet cream and the flavor added without detriment to keeping qualities.

Homologs of Diacetyl

According to Waser and Mohler (132) the next higher ketone homolog of ac_2, triketopentone ($CH_3 \cdot CO \cdot CO \cdot CO\,CH_3$), displays a more intense odor than ac_2. It possesses a penetrating and adhering aroma of ginger bread spice.

Prill *et al.* (97) noted that acetylpropionyl, acetyl-*n*-butyryl, acetyl-*iso*-butyryl, dipropionyl, acetyl-*n*-caproyl and di-*n*-butyryl resembled ac_2 in odor and certain other properties, but methylglyoxal and other alpha keto-aldehydes, as well as the alicyclic and aromatic diketones, differed distinctly from it in odor and also other respects. The various compounds showed similar behaviors in many of the reactions used for determination of ac_2. Methylglyoxal and glyoxal, however, could be distinguished from alpha diketones by means of the alpha-methylindole test. Certain homologs of ac_2 (acetylpropionyl and dipropionyl), as well as ac_2, could be determined colorimetrically as diammino-ferrous derivatives of their dioximes. When acetylpropionyl or dipropionyl was worked into salted butter, the effect on the flavor definitely suggested the effect of ac_2 but was less than with an equivalent amount of ac_2 and was less with dipropionyl than with acetylpropionyl. In butter, acetylpropionyl or dipropionyl contents, comparable to the ac_2 contents developed through use of cultures, were well retained over a considerable period at various temperatures. Distillates from ordinary butter cultures gave no evidence of the presence of higher homologs of ac_2 or amc; methylglyoxal also was absent.

Miscellaneous

Aschan (4) concluded that large quantities of butter are required to obtain the active constituents causing the aroma of butter and that a more profitable attack would be to synthesize the mono- and di-glycerides of the fatty acids which affect the senses of taste and smell.

Tapernaux (120) stated that numerous chemical products have been recommended for production of aroma in butter. Some of these are: Essence

of hazelnuts, essence of bitter almonds (benzoic aldehyde), essence of mirbane (nitro-benzene), coumarin, vanillin, butyric acid and its esters (monobutyrin, *iso*-propyl butyrate, etc.). He noted that none of the above products, either alone or in combination, duplicates the exact odor of butter and suggested that the best technic for developing the aroma of butter in a fatty material is to initiate a natural fermentation which permits development of the aromatic substances *in situ*.

Since lactic acid is odorless it cannot influence the aroma of butter. However, when present in appreciable amounts, it definitely affects the taste.

FACTORS INFLUENCING ACETYLMETHYLCARBINOL AND DIACETYL CONTENTS OF BUTTER

A series of factors related to the manufacturing procedure have an influence on the amc and ac_2 contents of butter and thus, presumably, on the flavor of the butter.

Influence of Strain of Butter Culture

Practical experience indicates that there are wide variations in the butter cultures used in different plants. In some cases cultures have definite defects, lack of flavor being the most common (43; p. 142), but even with normal cultures there are flavor variations (43; p. 102) of significance. These variations may not be interpreted the same by different persons, one culture in a series being preferred by one person and another by some one else. Because of variations in a culture from day to day, due to the acidities developed and other factors, the cultures in a series may not be rated the same on different days. In general, an experienced person can very accurately judge the value of a particular culture for development of flavor in butter.

Walts (131) compared nine commercial cultures and found there was no particular culture which was superior to the others as far as flavor of the butter was concerned. Knudsen (57) noted a close correlation between the aroma of butter and the aroma of the culture used.

Slatter and Hammer (117) reported an increase in the amc + ac_2 contents of salted and unsalted butter with an increase in the amount of culture employed; this suggested that the flavoring materials in the butter come largely from the culture rather than from changes in the cream during holding.

Krenn (58) added 5 per cent of three cultures containing 0.595, 0.71 and 3.98 mg. ac_2 per l., respectively, to three lots of cream. The cream was ripened and before churning contained 1.85, 1.012 and 2.42 mg. ac_2 per l., respectively. The amounts of ac_2 contributed by the cultures were 0.02975, 0.0357 and 0.199 mg., respectively, while the amounts formed in the cream were 1.82, 0.9763 and 2.22 mg., respectively.

Results obtained by Prill and Hammer (98) showed that, when culture was added to sweet cream, the cream-culture mixture contained approximately the amount of amc contributed by the cream and the culture, whereas the ac_2 content was regularly higher than the calculated amount. The authors suggested that incorporation of air during the mixing presumably made it possible for the organisms to effect considerable oxidation. After holding overnight at 36° to 44° F., the ac_2 had increased further, often more than doubling, and the amc also showed some increase but the percentage increase was not as great as with ac_2. No close relationship was noted between the ac_2 contents of the cultures and of the finished butter with churnings made by adding the cultures to sweet cream. Results obtained with culture and sour cream showed that more ac_2 was contributed to the cream-culture mixture by the neutralized and pasteurized cream than by the culture; amc was contributed in comparable amounts from the two sources. However, the culture supplied active organisms which made possible a further accumulation of the two compounds. Changes occurring in the ac_2 and amc contents were similar to those obtained with mixtures of sweet cream and culture.

In the trials of Hedrick and Hammer (45) on the ripening of sweet and sour cream for unsalted butter, the amc and ac_2 contents developed by different cultures varied rather widely in some instances and were rather uniform in others. When the same cultures were used in several trials they did not always rank the same on the basis of production of amc or ac_2, and the variations were not explainable on the basis of the slight differences in acidities of the ripened cream. Also, with the same cultures being used in several trials, each of them sometimes showed relatively high values and sometimes relatively low values.

Influence of Acidity of Cream

Investigations have clearly established that in a butter culture relatively large amounts of amc and ac_2 are only produced when considerable acid has developed (43; p. 114). This general relationship also applies in the ripening of cream. Because of it also, the amc and ac_2 contents of the original cream are significant only when the cream has developed some acid.

Tapernaux (120) stated that during the course of cream ripening acid develops and there appears a particular aroma due to the lactic acid organisms. He detected ac_2 in ripened cream; it was most evident when the ripening was not preceded by pasteurization. According to Kay (54) acid formation in cream is essential for aroma production since sweet cream does not contain amc. In the experiments of Barnicoat (6) butter made from cream ripened with culture showed amc + ac_2 contents which paralleled the rise in acid during the ripening.

Michaelian and Hammer (73) found the amounts of amc + ac_2 in sour cream delivered to butter plants was relatively high. They stated that this

was to be expected because of the wide distribution of flavor organisms and the favorable conditions provided by the acid cream for production of amc + ac_2.

Barnicoat (6) indicated that there is little amc + ac_2 developed when cream is ripened to the small extent common in the manufacture of mild-flavored butter. Most of the flavor-producing substances present in butter made from slightly ripened cream are not developed during the ripening but are added with the culture. This was demonstrated by experiments in which butter made from cream containing only 0.5 per cent culture, and in which no acid was developed, had a distinct aroma.

In the trials of Bouska (10) cream which had soured spontaneously for a moderate period, and which was churned without culture, produced butter with aroma. However, cream which had soured so long that amc and ac_2 had disappeared did not produce aromatic butter unless culture was added. Bunger (16) and also Mohr and Wellm (79) noted that butter made from sweet cream had much lower amc and ac_2 contents than butter made from sour cream. Makar'in (67) reported that the amc and ac_2 contents increased sharply during souring of cream; the same was true during souring of skimmilk.

Davies (24) stated that the rate of formation of ac_2 in cream can best be measured by calculating the ratio of ac_2 to acid (ac_2 as ppm. and acid as per cent lactic). When cream was ripened for 3 hours at 89.6° F. with a cheese culture the ratio was 3, with a butter culture it was 6 and with a *S. cremoris* culture it was 24; acidities were 0.25, 0.24 and 0.20 per cent, respectively. Incubating for longer periods showed that the ac_2 content of the *S. cremoris* culture increased more rapidly than that of the other two cultures with less increase in acid. The total ac_2 and amc in each culture increased roughly with acid, but at different rates. In 6 hours the ac_2 to amc ratio was 1:40 with the cheese culture, 1:20 with the butter culture and 1:18 with the *S. cremoris* culture. Davies (25) also noted that the most desirable property of a culture is that of forming the largest amount of ac_2 at a low acidity since in higher acid ranges it is the amc which is formed in largest amount; in general, at low acidities the ratio of ac_2 to amc is greater than at higher acidities.

According to Davies (25) when a culture is used to acidify cream, the titrable acidities of the cream which correspond to flavorless butter, mild-flavored butter, medium-flavored butter and full-flavored butter are 0.10 to 0.15, 0.15 to 0.25, 0.25 to 0.40 and 0.40 to 0.75 per cent, respectively. These figures apply to cream containing about 35 per cent fat. The cream serum acidities would be 0.13 to 0.20, 0.20 to 0.30, 0.30 to 0.50 and above 0.50 per cent, respectively. Various factors other than acidity which influence the degree of flavor development were listed as: (a) Distribution of the various types of lactic acid bacteria in the culture; (b) their virility under com-

petitive conditions in cream; (c) citric acid and oxygen contents of the cream; and (d) degree of biochemical and oxidation reactions occurring in the ensuing butter. Later, Davies (27) reported that the following titrable acidities in cream will ensure the various degrees of butter flavor: Mild flavor, 0.20 to 0.25 per cent; medium flavor, 0.25 to 0.35 per cent; and full flavor, 0.35 to 0.55 per cent. The corresponding ac_2 contents of lots of butter of ascending degrees of flavor were: Mild flavor, 0.5 to 1.5 ppm.; medium flavor, 1.5 to 2.5 ppm.; and full flavor, 2.5 to 4.0 ppm. These conditions referred to cream of 30 to 38 per cent fat, pasteurized by the flash method at 180° to 190° F., cooled and ripened at 65° to 70° F. to the desired acidity with butter culture. The author stated that if depth of flavor of butter is of importance, it is necessary to produce as much ac_2 as possible during the initial stages of cream ripening. That is, the flavor organisms must produce aroma before *S. lactis,* which produces acid only, depletes the cream of its dissolved oxygen. The ratio of ac_2 to amc was believed to depend on oxidation-reduction reactions occurring in the cream and these are governed, to an appreciable extent, by the oxygen tension in the cream.

Results of laboratory trials by Hedrick and Hammer (45) on sweet cream ripened to various acidities at 50° to 70° F. showed that as the acidity of the cream increased the ac_2 content increased in 29 (81%) of the 36 trials and the amc content in 32 trials (89%). Most of the increases were significant, but some of them were very small. Both ac_2 and amc increases were small in the pH range 6.5 to 5.5. In the remaining trials the ac_2 and amc contents showed changes other than regular increases as the acidity of the cream increased. Ac_2 contents of six lots of sweet cream with added culture held for a normal ripening period at 32° F. ranged from 0.12 to 0.28 ppm. (average 0.22 ppm.); the amc contents varied from 3.5 to 16.5 ppm. (average 10.6 ppm.). Three trials were carried out with neutralized sour cream ripened to various acidities at 58° to 62° F. Three acidities were used in each of two trials and four acidities were employed in one trial; in each trial a portion of the cream-culture mixture was held at 32° F. for the normal ripening period. An increase in acidity resulted in an increase in ac_2 and also in amc in each case. Increases in ac_2 were conspicuous and in two of the trials increases in amc were large. The ac_2 and amc contents of the three lots of neutralized sour cream with added culture held at 32° F. generally were higher than those of sweet cream with added culture, the average ac_2 content being 0.42 ppm. (from 0.35 to 0.55 ppm.) and the average amc content 22.1 ppm. (from 20.9 to 23.3 ppm). This relationship is explained by development of the two compounds during the souring of the cream.

In ten trials on a semi-commercial scale Hedrick and Hammer (45) ripened sweet cream (seven trials) and sour neutralized cream (three trials) to various acidities at 50° to 55° F. Three acidities were employed in three of the trials with sweet cream and two acidities were used in the

remaining trials. In two trials with sour cream, ac_2 and amc determinations were made just after pasteurization and again after addition of culture. With an increase in acid there was an increase in ac_2 in nine trials (90%) and in amc in seven trials (70%). Some of the increases were small. Ac_2 decreased in one trial (10%), the drop being from 1.88 to 1.49 ppm. with an increase in acid from 0.37 to 0.49 per cent. Amc decreased in three trials (30%), in one instance after a striking increase; two of the trials involved sour cream in which the citric acid may have been completely fermented. In general, as the ac_2 increased during the ripening of cream the amc also increased, but in some instances there was an increase in ac_2 and a decrease in amc. Commonly, increases in ac_2 and amc were in the same general proportion, but again there were exceptions.

The effect of ripening acidity on the score of unsalted butter was studied by Hedrick and Hammer (45). When the butter was held 1 week at 36° to 40° F., ripening cream to the higher of the two acidities used in each trial gave butter which scored 0.25 to 0.75 of a point higher than ripening to the lower acidity in five (71%) of the seven trials; there was no difference in score in one trial (14%) in which the acidities of the two lots of cream differed only 0.04 per cent; and in one trial (14%) the butter from the higher acid cream scored 0.25 of a point lower but the difference in the cream acidities was small, although both were relatively high (0.48 and 0.51%). After 1 month at 36° to 40° F., the butter made from the higher acid cream scored 0.25 to 0.5 of a point higher in three trials (43%), no difference in score was noted in two trials (29%) and the butter from the higher acid cream scored 0.5 of a point lower in two trials (29%). After 6 months at −10° to 0° F., ripening to the higher acidity gave butter which scored from 0.25 to 0.75 of a point higher in all trials.

Influence of Temperature of Ripening

Various investigations have dealt with the effect of ripening temperature on production of amc and ac_2 in butter cultures and similar studies have been carried out in connection with cream ripening.

Bunger (16) reported that cream allowed to sour at 62.6° F. had the highest content of amc and ac_2.

Wiley (135) inoculated sweet cream with 10 per cent culture and incubated it at 80° F. When ripened to pH 5, the cream contained about 0.8 ppm. ac_2 and 18 ppm. amc. Pasteurized sweet cream ripened with culture to pH 5 and then held 24 hours at 48° F. contained about 4.0 ppm. ac_2 and 90 ppm. amc.

Mohr and Wellm (79) noted that the ac_2 content of butter varied with the ripening temperature of the cream. Temperatures of 62.6° F., 50° F. and a combination of 62.6° and 50° F. were studied. Butter made from cream ripened at 62.6° F. contained 0.36 mg. ac_2 per kg. and 2.76 mg.

amc + ac_2; these were the largest amounts found. According to Mazé (71) the most favorable temperature for development of flavor and aroma substances in cream is 57.2° to 60.8° F.

Fabricius and Hammer (33) determined the amounts of amc + ac_2 in cream held overnight at 28° to 36° F. after adding 8 per cent culture, in cream held overnight at 42° to 51° F. after adding 8 per cent culture, and in cream ripened 1 hour at 70° F. and then cooled and held at 28° to 36° F. after adding 8 per cent culture. Results showed that the unripened cream held at 42° to 51° F. usually was higher in amc + ac_2 than the unripened cream held at 28° to 36° F. and contained about the same amounts as the ripened cream held at 28° to 36° F. Amounts of amc + ac_2 in cream held cold after adding culture 16 hours before churning and in cream to which culture was added at churning also were compared. The higher amc + ac_2 contents were found with addition of culture 16 hours before churning. Another series of comparisons involved cream held at a low temperature for 3, 6, or 16 hours after adding culture; there were both increases and decreases in amc + ac_2 contents as a result of the holding. Effects of holding for 64, 70, 88 or 112 hours also were variable. Immediately after adding culture the amc + ac_2 contents of the different portions in a trial were essentially the same.

Laboratory tests on the effect of ripening temperature on the ac_2 and amc contents of cream were made by Hedrick and Hammer (45). In 18 trials sweet cream was ripened at 50°, 60° and 70° F. Ripening periods varied from 5 to 45 hours, depending on the temperature and the degree of acid desired. Ac_2 and amc determinations were made soon after the desired acidity was reached. The highest ac_2 content developed at 50° F. and the lowest at 70° F. in 14 of the trials (78%); the same relationship occurred with amc in 11 trials (61%). Both ac_2 and amc production were highest at 50° F. and lowest at 60° F. in two trials (11%). The highest ac_2 content was produced at 60° F. and the lowest at 70° F. in one trial (6%); the same relationship occurred with amc in five trials (28%). Ac_2 production was highest at 70° F. and lowest at 60° F. in one trial (6%). Variations in amc production at the three temperatures were small and definitely less on a percentage basis than variations in ac_2 production. Four trials were carried out on a semi-commercial scale; three involved sweet cream and one involved neutralized sour cream. Ac_2 production in cream ripened at 62° F. was larger than in cream ripened at 70° F. in two trials (50%) and smaller in two trials (50%). Amc production showed similar variations. Differences in production of ac_2 and amc in a comparison commonly were small. In three trials, 0.1 per cent citric acid was added to the cream and the cream agitated during ripening overnight to an acidity of 0.45 per cent. Ac_2 and amc production was much greater in each trial than in the cream ripened at either 62° or 70° F.

Effect of ripening temperature on the score of the resulting unsalted butter was studied by Hedrick and Hammer (45). With butter held 1 week at 36° to 40° F., ripening of the cream at 62° F., rather than 70° F., gave a score increase of 0.25 to 0.75 of a point in two trials (50%), no difference in score in one trial (25%) and a score decrease of 1 point in one trial (25%). With butter held 1 month at 36° to 40° F., ripening at 62° F. gave a score increase of 0.5 to 0.75 of a point in each of three trials (100%). With butter held 6 months at −10° to 0° F., ripening at 62° F. gave a score increase of 0.5 of a point in each of two trials (50%) and a score decrease of 0.75 and 1.0 point in two trials (50%).

Hoecker and Hammer (47) found that on holding cream plus culture 16 hours at 40° F., the ac_2 and amc contents increased when butter culture, *S. diacetilactis* or *S. citrophilus* was used; decreases often occurred with *S. citrovorus* or *S. paracitrovorus;* usually little or no change occurred with an unidentified organism or *S. aromaticus.*

Influence of Fat Content of Cream

Variations in the fat contents of different lots of cream are large enough to be of importance from the standpoint of cream ripening and butter manufacture. With a high fat content the serum content is small, and a certain percentage of butter culture, based on the cream, has a greater effect in lowering the pH, etc., than when the fat content is low and the serum content correspondingly high. On the other hand, with a high serum content the percentage of citric acid in the cream is larger.

Orla-Jensen (85) stated that since the lactic acid bacteria do not act on butterfat they must produce the characteristic butter aroma and acid from the other constituents of the milk; therefore, the richer the cream, the less aroma will be formed therein.

Barnicoat (7) investigated the effect of the growth medium on development of ac_2 and amc. Experiments made with milk and cream steamed for 30 to 40 minutes, allowed to cool and inoculated with 0.5 per cent culture showed that there was no essential difference, with due allowance for the fat content of the cream, in the action of culture in skimmilk, whole milk and cream.

Ac_2 and amc contents of butter churned from whole milk and from cream of varying fat contents were determined by Mohr and Wellm (79). Butter made from 40 per cent cream contained the most ac_2, while butter made from 20 per cent cream and that made from whole milk were about equal in ac_2 contents. Amc contents of the lots of butter increased with increasing fat contents of the cream. Bunger (16) noted that on churning sour whole milk, 20 per cent cream and 40 per cent cream, the ac_2 content was highest in the butter from the 40 per cent cream; butter made from the whole milk and that made from 20 per cent cream contained practically the same amounts of ac_2.

Bogdanow (9) recommended an increase in the fat content of the cream in order to obtain increased aroma. He stated that aroma is absorbed by the fat.

Influence of Addition of Citric Acid to Cream

Since citric acid is the important source of flavor materials in butter cultures (43; p. 116) and the amount in milk is comparatively small (43; p. 102), this acid or its sodium salt sometimes is added to milk intended for culture. Such an addition also would be expected to have an effect in the ripening of cream.

Preliminary experiments from the Union of South Africa (3) indicated that cream churned with added citric acid gives butter of better flavor than that from untreated cream. Orla-Jensen (85) reported that autolysed yeast extract added to cream did not afford a sure means of increasing the aroma of butter but addition of citric acid gave more promising results. Templeton (121) found that addition of citric acid or sodium citrate to butter culture, to the cream used for butter or to both gave butter with a more pronounced flavor and aroma. Addition of 0.2 per cent citric acid, or its equivalent of sodium citrate, to the culture alone had a slight effect. The effect was more pronounced when the same proportion of citric acid or citrate was added to the cream.

Khubchandani (55) noted that addition of citric acid or sodium citrate to cream resulted in butter with increased aroma but poorer keeping qualities. Hunziker (48; p. 364) concluded that, in the absence of more favorable experimental data, addition of citric acid to cream appears to be of doubtful merit; he suggested avoiding the expense until more convincing knowledge of the merits of the addition is available.

Experiments involving addition of citric acid to cream at the time of inoculating with culture were reported by Hedrick and Hammer (45). In 14 laboratory trials sweet cream was ripened with and without added citric acid to about the same acidity at temperatures from 54° to 60° F.; 0.05 per cent acid was added except in one trial in which 0.10 per cent was used. Addition of citric acid gave a higher ac_2 content in the cream in all the trials (100%) and a higher amc content in 12 trials (86%). With both ac_2 and amc some of the increases were very small. In eight trials neutralized sour cream was ripened with and without citric acid to about the same acidity at temperatures from 58° to 63° F. In six trials (75%) added citric acid resulted in an increase in the ac_2 content. In the four trials involving three amounts of acid, the increases were roughly in proportion to the amount added; in one trial (12.5%) 0.05 and 0.10 per cent added citric acid did not increase the ac_2 content but 0.15 per cent did, and in one trial (12.5%) 0.10 and 0.15 per cent citric acid gave the same ac_2 content. Added citric acid regularly increased the amc content and, in the trials in which three amounts were used, the increases were roughly in proportion to the amount

added. Data were obtained on seven semi-commercial trials in which cream was ripened with and without added citric acid (0.05%) to about the same acidity at temperatures from 50° to 54° F. Added citric acid increased the ac_2 content in six comparisons (86%); the increases varied widely, with three of them being 0.20 ppm. or less. The one decrease in ac_2 content was very small, being 0.13 ppm. Added citric acid increased the amc content in three comparisons (43%), the increases ranging from 12.8 to 34.8 ppm. In one trial (14%) the amc content was the same with and without added citric acid. In the three comparisons (43%) in which addition of the acid resulted in a decrease in amc content, the decreases ranged from 4.0 to 15.8 ppm.

Hedrick and Hammer (45) studied the effect of adding 0.05 to 0.10 per cent citric acid to cream on the score of the resulting unsalted butter. With butter held 1 week at 36° to 40° F., addition of citric acid increased the score 0.25 to 0.50 of a point in four trials (57%) and there was no difference in score in three trials (43%). With holding 1 month at 36° to 40° F. the addition gave a score improvement of 0.25 to 0.75 of a point in six trials (86%) and no difference in score in one trial (14%). With holding 6 months at −10° to 0° F. the addition resulted in an increase in score of 0.5 to 0.75 of a point in six trials (86%) and a score decrease of 0.5 of a point in one trial (14%).

Influence of Aeration of Cream

The oxygen supply is a factor in the production of amc and ac_2 in butter cultures (43; p. 118) and, accordingly, should be important in flavor development in cream. However, aeration may have an undesirable influence on the flavor of some of the milk constituents because of their tendency to oxidize.

According to Orla-Jensen (85) aeration of cream may possibly promote production of aroma; it increased the volatile acidity of cream. Davies (23) noted that most of the ac_2 in cream is formed while there is considerable atmospheric oxygen in solution.

Virtanen and Tarnanen (129) considered that the fact ac_2 is formed only when oxygen acts as the hydrogen acceptor should be considered in butter manufacture. They suggested that cream be ripened under as aerobic conditions as possible and that churning be carried out so as to ensure a maximum content of air in the butter. (See also 128.) Virtanen (128) found that passing sterile air into cream during ripening had a very favorable effect on the aroma of fresh butter, the ac_2 content being increased considerably. Makar'in (67) stated that the upper layers of souring cream are richer in ac_2 and amc than the lower layers.

Davies (24) reported that formation of amc was favored by a low oxygen content. Later, he (25) indicated that when a culture was added to cream,

the important factor was the rate at which the aroma-producing organisms proliferated before the true lactic acid bacteria induced a low oxygen content. Ac_2 was found in cream in greatest amounts when there still was some free oxygen present.

It was noted by Prill and Hammer (98) that when sweet cream and aerated-pressure (p. 89) or modified aerated-pressure (p. 88) culture were mixed, analyses did not indicate an increase in ac_2 during the mixing but sometimes suggested a slight decrease, especially in the case of aerated-pressure culture. During the holding of the cream-culture mixtures overnight and during churning, the organisms usually produced additional ac_2 and amc; although in most cases the actual increment in ac_2 was as great (or greater) than when regular butter culture was used, the percentage increase was not because of the relatively large amount of ac_2 originally present. It appeared that if the cream had been churned soon after addition of culture, aerated-pressure or modified aerated-pressure culture probably would show a greater practical advantage over regular butter culture.

Laboratory trials on the effect of agitation during the ripening of cream on the ac_2 and amc contents were reported by Hedrick and Hammer (45). In nine trials (seven with sweet cream and two with neutralized sour cream) cream was ripened with and without agitation at 60°, 63° or 70° F. At 60° or 63° F. the cream was agitated three times during the ripening while at 70° F. it was agitated every hour for 3 or 4 hours. In each trial the ac_2 and amc contents of the agitated cream were much higher than those of the unagitated control. Essentially the same acidity developed with and without agitation. Effect of agitation with added citric acid was studied in six trials (four with sweet cream and two with neutralized sour cream); 0.10 per cent citric acid was used and the ripening temperature was 60°, 63° or 70° F. As without addition of citric acid, agitation greatly increased both the ac_2 and amc contents but had no appreciable effect on acid development. Five trials were carried out on a semi-commercial scale (three with sweet cream and two with neutralized sour cream). Again the ac_2 and amc contents were greatly increased by the agitation whereas the acid development was not significantly influenced.

In a study of the effect of agitation during ripening on the score of the resulting unsalted butter after 1 week at 36° to 40° F., Hedrick and Hammer (45) found that agitation of the cream gave a score increase of 0.25 to 0.75 of a point in three (60%) of the five trials and no difference in score in two trials (40%). With holding of the butter 1 month at 36° to 40° F., agitation gave a score increase of 0.5 to 0.75 of a point in two trials (40%), no difference in score in two trials (40%) and a score decrease of 0.25 of a point in one trial (20%); with holding 6 months at −10° to 0° F., agitation resulted in a score increase in four trials (80%) and no difference in one trial (20%).

Hoecker and Hammer (47) reported that immediately after mixing the ac_2 contents of cream with added butter culture were sometimes higher and sometimes lower than the theoretical amounts calculated from the ac_2 contents of the cream and culture; in most trials the amc contents were about the same as the theoretical values but in some instances were higher.

Influence of Neutralization and Pasteurization of Cream

Neutralization and pasteurization of cream constitute the primary treatments of much of the cream intended for butter, neutralization now being employed even with some of the sweet cream used for butter. In the investigations of the effect of these processes on the amc and ac_2 contents of cream, there has been some variation in the results, probably because of variations in the types of cream used and in the neutralization and pasteurization procedures.

Barnicoat (7) found that neutralization and pasteurization of cream caused no notable decrease in ac_2 but a distinct decrease in amc. Destruction of amc was correlated with the neutralization point of the cream and was similar with the two types of pasteurizers used; it was thought to have some bearing on the opinion, frequently expressed, that butter made from over-neutralized cream often is deficient in flavor.

Davies (24) noted that cream which had developed certain amounts of amc and ac_2 did not yield butter having flavor or aroma when the cream was neutralized to 0.10 to 0.15 per cent acid and flash pasteurized. Disappearance of amc and ac_2 from the cream was explained on the basis of volatility of the aroma constituents and absence of oxidation of amc in the butter. Examination of many samples of neutralized cream butter after 6 months in storage revealed no trace of ac_2 or amc + ac_2 by the usual analytical methods. Later Davies (26) stated that butter made from neutralized cream possesses no butter flavor; if flavor is required, the pasteurized and cooled cream is ripened by addition of culture to different acidities, depending on the degree of flavor desired.

According to Prill and Hammer (98) neutralized and pasteurized cream frequently had a lower ac_2 content than the raw cream. With cream having an original acidity of 0.5 to 0.6 per cent, it was found that after preliminary neutralization to 0.25 per cent acid, or during the first part of the heating, the ac_2 content fell to a very low value and the amc content commonly decreased slightly. The compounds may have been reduced to 2,3-butylene glycol. When the cream had been heated to pasteurizing temperature, and also in subsequent examinations, the ac_2 contents showed very significant increases. The amc content showed an increase when the cream had reached pasteurizing temperature and then remained fairly constant in the subsequent examinations. The authors stated that the increase in ac_2 may possibly have been caused by non-biological oxidation but the increase in

amc through non-biological reactions seemed improbable. Net result of the cream processing was a decrease in ac_2 content and relatively little change in amc content.

Influence of Certain Metallic Salts

Mohr and Arbes (77) noted that when milk or cream was treated with certain metallic salts and then soured and stored for approximately 20 hours, or allowed to sour during such storage, the ac_2 content was increased over that of the control lot. The salts tested, in order of increasing effectiveness, were cupric chloride, manganese chloride, ferric chloride and a combination of all three; they were added in amounts of 0.5 to 2.0 mg. per l. of milk or cream. Addition of the salts to milk or cream immediately before determining the ac_2 was without effect on the results.

Influence of Churning

Because of the agitation of the cream and the butter granules in the presence of air, churning would be expected to have an effect on the ac_2 contents of the cream and the resulting butter and perhaps on the amc contents.

Tapernaux (120) stated that the butter formed by agglomeration of fat globules from the cream during churning seems to retain the aromatic substances, and the aroma of butter continues to increase in intensity throughout the days which follow manufacture; the butter also contains fermentable substances and ferments. According to Davies (22) ac_2 probably is not formed until butter is churned and stored. Bunger (16) noted that the ac_2 content of cream increased during churning.

Mazé (71) considered that churning must be regarded as an additional aerobic fermentation, its object being to oxidize the reductive products of the anaerobic lactic acid fermentation and to produce the flavor characteristic of fresh, first-quality butter. Results obtained by Mohr and Wellm (79) indicated that the largest contents of ac_2 and amc were obtained in fresh butter at a churning temperature of 57.2° F.

Virtanen (128) stated that churning of cream in a churn filled with some indifferent gas is obviously not suited to production of butter with a rich aroma. He suggested that churning should be carried out in such manner as to ensure a maximum content of air in the butter since this promotes formation of amc and finally ac_2.

The analyses of Prill and Hammer (98) showed an increase in ac_2 content during churning. The authors noted that the amc content would not be expected to change greatly in the short period involved.

Influence of Washing Butter with Water

Because of the solubility of amc and ac_2 in water, the washing of butter would be expected to remove some of these materials from it.

In 1893, Conn (20) noted that the flavor of butter was very much more prominent without thorough washing than with it. If the butter was washed long enough, all of the aroma could be washed away.

Leitch (60) indicated that much of the aroma of butter is lost during the washing. He recommended washing butter with serum prepared by fermenting pasteurized skimmilk with culture, removing the curd and filtering the liquid through sterile linen. Testoni and Ciusa (123) found that when butter was washed four times it no longer contained ac_2; when washed only twice it contained the compound.

According to results obtained by Barnicoat (6) no extraction of amc + ac_2 appeared to take place during the washing of butter. Later he (7) reported the loss of amc + ac_2 by the washing of butter was relatively unimportant.

Krenn (58) noted that butter wash water contained considerable ac_2. Bunger (16) removed the greater part of the amc and ac_2 from butter granules by washing them three times. Makar'in (67) reported that washing butter greatly reduced the ac_2 content. In the analyses of Mohr and Wellm (79) unwashed butter contained 1.69 mg. ac_2 per kg. and 9.30 mg. amc + ac_2 whereas washed butter contained 0.86 mg. ac_2 and 3.78 mg. amc + ac_2. According to Pont (94) approximately four-fifths of the amc + ac_2 originally present is removed by the buttermilk and the washing of the butter.

Mohr and Arbes (77) washed butter with water containing 0.5 to 1.5 mg. per l. of various salts of iron, manganese and copper; they concluded that the salts had no effect on the ac_2 content of the butter.

Prill and Hammer (98) analyzed several samples of butter wash water and found only a fraction of a mg. of amc + ac_2 per kg. They stated that while the total amc + ac_2 in wash water may be significant, compared to the amount present in the butter, it is difficult to differentiate between material which comes from the film of buttermilk remaining between the butter granules and that which might possibly be removed from the granules themselves. If amc and ac_2 are both held entirely in the serum droplets in the same manner, they should be retained in the butter in the same proportion as they exist in the churning mixture at the time the butter is formed, and any possible washing out of the compounds should also be in this proportion.

Partition of Acetylmethylcarbinol and Diacetyl During Churning

When cream is churned, some of the amc and ac_2 goes into the butter and some into the buttermilk. This general relationship has led to various studies on the partition of the compounds during churning. Presumably, from the standpoint of butter flavor, it would be better if all the amc and ac_2 present in the cream were retained in the butter.

Comparative amounts of acetylmethylcarbinol and diacetyl in cream and butter. Barnicoat (6) calculated that about one-fifth of the amc + ac_2

in cream is carried into the butter. Michaelian and Hammer (73) noted that butter regularly contained much less amc + ac_2 than the cream from which it was churned. With four lots of butter the amc + ac_2 was not measurable with the gravimetric method then commonly in use but with the other 52 lots in the series the ratios of amc + ac_2 contents of the cream to those of the butter ranged from 1 : 0.032 to 1 : 0.218. The ratios were not correlated with the amount of amc + ac_2 in the cream or the acidity of the cream.

Results obtained by Mohler and Herzfeld (76) indicated that cream from which the butter was churned contained about three times more ac_2 than the resulting butter. Davies (23) reported that only about one-fifth of the amc + ac_2 in cream appeared in the butter and about one-fourth of the ac_2. Well ripened cream (0.6 per cent acid) containing 5 to 10 ppm. ac_2 and 100 to 250 ppm. amc yielded butter containing 1 to 2 ppm. ac_2.

According to Barnicoat (7) the percentage of ac_2 retained by butter varies from 0 to 33 per cent, the extremes occurring with neutralized creams. Average retention in the butter was 15 per cent of the amount originally in the cream. The ratio of ac_2 in butter to ac_2 in cream (30 to 40 per cent fat) was 0.4. The ratio of ac_2 to amc in "mild starter" butter examined 1 day after manufacture was greater than in cream; the average ratio of ac_2 to amc in 11 lots of cream was 0.07 while in the butter the ratio was 0.11.

Brioux and Jouis (14) found that butter contained less ac_2 than the cream from which it was churned. Bunger (16) noted that from one-fourth to one-sixth of the ac_2 and from one-fifteenth to one-sixteenth of the amc in the cream was present in the resulting butter.

Barnicoat (7) reported that when amc, as the synthetic compound, was added to pasteurized cream there was, in the absence of butter culture, a loss before churning. Results obtained on 12 churnings showed that from 7 to 22 per cent (average 14%) of the amc in the cream was retained in the butter.

Davies (24, 25, 26) noted that the ratio of ac_2 in cream to that in butter was approximately 4 : 1; the ratio of amc + ac_2 in cream to that in butter was between 4 : 1 and 5 : 1.

Analyses made by Mohr and Wellm (79) on washed and worked butter showed that the butter had only one-fourth to one-sixth of the ac_2 content of the cream and from one-fifteenth to one-sixteenth of the amc content.

Krenn (58) determined the amounts of ac_2 in butter obtained from cream which contained 1.012 and 2.42 mg. ac_2 per l., respectively. The butter contained 0.357 and 0.00 mg. ac_2 per kg., respectively. Krenn concluded that only a small part of the ac_2 formed during the ripening of cream is present in the resulting butter. Brioux and Jouis (14) found that cream containing 95.2 mg. amc per kg. yielded butter containing 42.4 mg.; the same cream contained 1.92 mg. ac_2 and the resulting butter 1.50 mg. ac_2.

According to Davies (25) the amount of ac_2 in butter is roughly 10 per cent of that in the cream. The amount in the finished butter was considered to be influenced by the amount of washing and the fraction of the moisture accounted for by the buttermilk droplets which, in turn, depends on the degree of working. The author further stated (27) that the distribution of ac_2 in butter is roughly proportional to the ratio of butter serum to buttermilk.

Results of Hoecker and Hammer (46) showed that only small percentages of the amc and ac_2 in a cream-culture mixture were retained in the butter, the remainder being in the buttermilk; the percentage retention was essentially the same with various cultures, although with each culture there was considerable variation from one churning to another.

Hedrick and Hammer (45) reported that with 74 semi-commercial churnings of unsalted butter, the minimum, maximum and average ratios of the ac_2 contents of the ripened cream and of the corresponding butter were 1:0.142, 1:0.709 and 1:0.352, respectively; the corresponding ratios for amc were 1:0.059, 1:0.683 and 1:0.217, respectively. The investigators also noted that the ac_2 and amc contents of a series of lots of butter sometimes did not follow the same order as the contents of the lots of cream from which the butter was churned. The irregularities were of all possible types. In some cases the ac_2 or amc content of a lot of butter was lower than that of an earlier lot in the series, although the content of the cream was higher. In other cases a value for ac_2 or amc was lower than the values on the lots preceding and following it in the series, even when there was no drop in the value for the cream. Still other types of variations occurred. The ac_2 contents of the semi-commercial churnings of butter ranged from 0.16 to 1.46 ppm., while the amc contents varied from 2.9 to 24.0 ppm. The highest values for both ac_2 and amc were obtained on a lot of butter made from cream ripened with agitation after addition of 0.1 per cent citric acid.

Hoecker and Hammer (47) stated that, in butter made with butter culture, amc and ac_2 are derived largely from the culture added to the cream and from fermentation of citric acid during holding or ripening of the cream.

Comparative amounts of acetylmethylcarbinol and diacetyl in cream and buttermilk. In 1893, Conn (20) noted that, after churning, nearly all the flavor produced during the ripening of cream was contained in the buttermilk. Butter aroma, therefore, was considered due to changes in some constituent of the cream other than fat.

Hammer (40, 41) stated that, when cream was churned, much of the amc and ac_2 in it was carried into the buttermilk so that the buttermilk contained more amc and ac_2 than the cream. Michaelian and Hammer (73) found that fresh buttermilk regularly contained more amc + ac_2 than the cream from

which it was obtained. The ratios of amc + ac_2 in the cream to that in the buttermilk ranged from 1:2.0 to 1:3.1. In a series of churnings involving cream of various acidities, the ratios ranged from 1:1.1 to 1:2.1.

According to Davies (23) aeration of cream during churning, and the consequent readjustment of the ratio of ac_2 to amc in the buttermilk, causes the buttermilk to contain more ac_2 than ripened cream. An increase in the acidity of the buttermilk resulted in a decrease in the ac_2 content.

Bunger (16) found that buttermilk contained from 2.4 to 4.0 times more ac_2 than the sour cream before churning. Results obtained by Davies (24) indicated that the ratio of ac_2 in buttermilk to that in the original cream varies from 2:1 to 3:1, depending on the fat content of the cream and the ratio of ac_2 to amc in the cream.

Krenn (58) determined the amount of ac_2 in buttermilk obtained from cream which contained 1.012 and 2.42 mg. ac_2 per l., respectively, before churning. The buttermilk contained 1.250 and 4.098 mg. per l., respectively. Buttermilk having an initial ac_2 content of 4.59 mg. per l. showed 4.53 mg. after holding 8 hours and 4.65 mg. after holding 24 hours. This increase was believed due to the lactic acid and aroma-forming bacteria which were present in large numbers.

Makar'in (67) noted that the ac_2 content of buttermilk was greater than that of the cream before churning. Results obtained by Mohr and Wellm (79) showed that buttermilk contained 2.4 to 4.0 times as much ac_2 as the cream before churning. Brioux and Jouis (14) stated that after churning the ac_2 and amc in fermented cream were contained largely in the buttermilk. Fermented cream containing 95.2 mg. amc per kg. yielded buttermilk containing 195.3 mg.; the same cream contained 1.92 mg. ac_2 and the resulting buttermilk contained 3.10 mg.

Comparative amounts of acetylmethylcarbinol and diacetyl in butter and buttermilk. van Niel *et al.* (126) found ac_2 in a sample of buttermilk having an exceptionally fine aroma and a high amc content. According to Hammer (41) there is a concentration of amc and ac_2 in the buttermilk rather than in the butter.

In order to check the accuracy of his investigation, Krenn (58) attempted an ac_2 balance. In one trial 10 kg. of cream (21% fat) produced 2.42 kg. of butter and 7.58 l. of buttermilk. The cream (9.88 l.) contained 10.0 mg. of ac_2, the resulting butter contained 0.86 mg. and the buttermilk (7.58 l.) contained 9.15 mg. Therefore 10.01 mg. of ac_2 was obtained in all, or 0.01 mg. more than was present in the cream. In another trial 10 kg. of cream (24.5% fat) produced 3.06 kg. of butter and 6.94 l. of buttermilk. The cream (10 l.) contained 24.2 mg. of ac_2 and the resulting butter contained none; the buttermilk (6.94 l.) contained 28.4 mg. or 4.2 mg. more than the original cream. This result was explained by a conversion of amc in the

cream to ac_2 by the carbonic acid present. To clarify this point 2 l. of sour cream containing 4.06 mg. of ac_2 was churned in a hand churn. The buttermilk was drawn completely and the resulting butter was washed three times with water. The buttermilk contained 6.14 mg. of ac_2, the first wash water 3.16 mg. and the two succeeding wash waters none. The resulting butter contained 0.3 mg. ac_2. In the trial, 5.54 mg. of ac_2 more than was present in the cream was accounted for. It appeared that ac_2 actually was formed during the churning process by oxidation of amc.

Results obtained by Alberti (1) indicated that considerable amc and ac_2 remained in the buttermilk.

Prill and Hammer (98) noted that certain general relationships applied to the distribution of amc and ac_2 between the butter and the buttermilk in all the churnings studied. The estimated mg. ac_2 in the buttermilk and the butter derived from 1 kg. of churning mixture agreed fairly well with the content of the partly churned mixture and the estimated mg. amc + ac_2 in the two products agreed fairly well with the content just before churning. The ac_2 contents of the butter were very nearly one-fifth those of the corresponding buttermilk. Since butter contains one-fifth serum, it would appear that ac_2 is contained entirely in the serum of butter. However, the amc + ac_2 content of the butter was, in most cases, only about one-tenth that of the buttermilk. The average of the estimated percentages of ac_2 in the churning mixtures that were retained in the butter was 8.8 per cent, and the corresponding value for amc + ac_2 was 4.5 per cent.

Partition of acetylmethylcarbinol and diacetyl between aqueous and fatty constituents of cream and butter. Orla-Jensen (85) stated that since the aroma of butter is produced outside the fat globules, it might be expected to wash out as easily as the lactic acid, but this is not the case. Isigny butter, which had a stronger aroma than any other make, was subjected to a very thorough washing during its manufacture. The explanation given was that butterfat can absorb essential oils and other odoriferous substances, both pleasant and unpleasant, and the aroma of the cream therefore passes into the fat globules. It was thought that the greater the proportion of fat globules present, the less aroma would be available for each globule and experience has shown that a high fat percentage is not conducive to production of an aromatic butter.

King (56) found a slow diffusion of ac_2 between the fat and serum phases of butter, ac_2 being dissolved in the fat fraction as well as in the serum. He believed that a slow diffusion of ac_2 from the serum to the fat occurred during storage.

Tapernaux (120) indicated that, during the course of cream fermentation, ac_2 is formed in the water and in the fat and divides itself between the serum and fat so that a part is eliminated in the buttermilk and also in the

wash water. Hammer (40, 41) reported that the amc and ac_2 in butter is mostly in the serum and only very little is in the fat.

According to Barnicoat (6) the final concentration of amc + ac_2 in butter is dependent on the amount of buttermilk retained. He believed that the compounds probably are held in the aqueous portion and are possibly absorbed on the protein rather than dissolved in the fat.

Michaelian and Hammer (73) found the amount of amc + ac_2 in the serum of butter was higher than the amount in the fat. Makar'in (67) stated that ac_2 in butter exists in the butter plasma which was considered to be made up of about one-half buttermilk. In one of eight trials, traces of ac_2 were found in the fat itself.

Davies (24, 25, 27) considered that since the vapor pressure of ac_2 is higher than that of amc a greater amount of ac_2 than of amc would be expected in the fat phase of cream. The amount of ac_2 in the fat, however, was less than the amount in the aqueous phase of butter. The author noted that a considerable amount of both ac_2 and amc occurs in the fat phase.

Mohr and Wellm (79) determined the amounts of amc and ac_2 in butter, butterfat and butter serum which was obtained by careful melting at 113° F. Butter containing 0.56 mg. ac_2 and 14.84 mg. amc + ac_2 per kg. had 0.52 mg. ac_2 and 5.14 mg. amc + ac_2 per kg. in the fat. Butter serum contained 1.24 mg. ac_2 and 61.1 mg. amc + ac_2 per l.

Virtanen (128) stated that ac_2 obviously is dissolved in the water portion of butter and is distributed between the buttermilk and the butter in the same proportions as water.

Results of Barnicoat (7) showed a higher ratio of ac_2 to amc in butter than in the cream from which it was churned. He considered that this observation might indicate a partition of the ac_2 with the fat but the data pointed to an actual development of amc in the butter. Neither ac_2 nor amc was concentrated in the fat during butter manufacture. Makar'in (68) stated that amc and ac_2 are not absorbed by the fat globules but are contained in the aqueous portion of butter. He believed that the aroma of butter was influenced by the concentration of aqueous constituents of the cream retained in the butter; as a rule this represented about 60 per cent of the total moisture content of the butter.

Hoecker and Hammer (47) studied the distribution of ac_2 and amc between "Wesson" oil and water or brine. In the mixtures the water or brine regularly contained higher concentrations of ac_2 and amc than the oil, the differences being greater with amc than with ac_2. The percentage of ac_2 in a mixture that was contained in the oil was increased by sodium chloride in the water. The concentration of ac_2 in a mixture did not affect the percentage in the oil. Concentrations of amc in the oil were very low and the different concentrations in the mixtures gave essentially the same percentages in the oil. Studies made on the distribution of ac_2 and amc between

butterfat and water or brine showed that the water or brine regularly contained higher concentrations of the compounds than the fat; differences were greater with amc than with ac_2. As the concentration of ac_2 in a mixture increased, the percentage in the fat did not change appreciably, while as the concentration of amc in a mixture increased, the percentage in the fat decreased. Studies on the distribution of ac_2 and amc between the fat and serum of unsalted and salted butter showed that the serum of butter contained higher concentrations of ac_2 and amc than the fat, with the greater differences again involving the amc. Averages of the percentages of ac_2 and amc contained in the fat were smaller with unsalted than with salted butter. The percentage of ac_2 contained in the fat was independent of the concentration in the butter, whereas the percentage of amc usually decreased somewhat as the concentration in the butter increased. Addition of a solution of ac_2 or a distillate of butter culture to salted butter resulted in essentially the same distribution of ac_2 as when the butter was made from cream containing butter culture. The amounts of ac_2 and amc in butter, as calculated from its composition and analyses of the fat and serum, agreed fairly closely with the determined amounts. In unsalted butter ac_2 and amc concentrations in the serum ranged from 0.25 to 3.55 ppm. and from 4.85 to 119.16 ppm., respectively, and those in the fat varied from 0.19 to 1.11 ppm. and from 0.88 to 6.86 ppm., respectively. The fat contained from 44.4 to 75.0 per cent of the ac_2 and from 26.2 to 46.8 per cent of the amc in the butter. In salted butter ac_2 and amc concentrations in the serum ranged from 0.07 to 2.00 ppm. and from 1.72 to 59.00 ppm., respectively, while those in the fat varied from 0.02 to 0.92 ppm. and from 0.29 to 4.87 ppm., respectively. Of the total ac_2 and amc in the salted butter, the fat contained from 48.5 to 78.4 per cent and from 23.6 to 46.0 per cent, respectively. The investigators concluded that comparative solubilities in the fat probably explain why with ac_2 the concentration in butter does not affect the percentage of the total that is retained in the fat, while with amc an increase in the concentration in butter decreases the percentage of the total that is retained in the fat. The low solubility of amc was believed to limit the amount taken up by the fat. Variations among samples of butter in the percentages of total ac_2 or amc that were contained in the fat were thought to be due to several factors, such as composition of the butter, physical state of the fat, churning procedure and degree to which water is dispersed in the butter; also, analytical errors involved in determining very small quantities of the compounds may be of minor significance. It also was concluded that since large percentages of the ac_2 and amc in cream at churning are removed with the buttermilk, higher concentrations of the compounds would be expected in the serum of butter than in the fat. Although concentrations in the fat were relatively low, the percentages of the compounds in butter that were contained in the fat were comparatively high because butter contains ap-

proximately 80 per cent fat. Partitioning of ac_2 and amc between the serum and fat was thought to reach an equilibrium in a relatively short time.

Influence of Reworking Butter

Reworking butter has an effect on the action of micro-organisms in it, including the butter culture types (64).

Barnicoat (7) stated that butter which develops off flavors during cold storage usually is considerably improved by reworking. This improvement has been thought to be due to formation of ac_2; results of the author, however, showed a slight decrease in ac_2 on reworking butter.

Influence of Storage of Butter

During the holding of butter there are various possibilities in connection with changes in the amc and ac_2 contents. The compounds may disappear through action of organisms or through chemical reactions. Also, under suitable conditions, they may be formed, particularly through action of the normal butter culture organisms; these organisms are much more active in unsalted than in salted butter (81).

Salted butter. Testoni and Ciusa (123) found that the amount of ac_2 in butter decreased on holding and that ac_2 did not develop in butter originally containing none. Tapernaux (120) stated that butter having an intense aroma tends to lose aroma as the holding period is extended and the aroma may even disappear. He suggested that the ac_2 is transformed into amc by reduction and then into 2,3-butylene glycol. Loss of aroma also was explained by the volatility of ac_2. Davies (22) reported that ac_2 is lost more rapidly in butter of higher acidity.

According to Barnicoat (6) when butter was made from slightly ripened cream, with or without butter culture, the amc + ac_2 content remained fairly constant throughout the storage (193 days at 14° to 17° F.). Butter made from cream which had been slightly ripened with culture showed some conversion of amc to ac_2; however, in two samples less ac_2 was present at 6 months than at 3 months. All the butter made with culture contained considerable amc + ac_2 (0.7 to 2.3 ppm.). The ac_2 disappearing from butter during storage was considered lost through oxidation to simpler substances. Decomposition of ac_2 was greater in butter made without culture than in butter made with culture. In butter made with culture, a certain amount of ac_2 was lost and considerable of the original ac_2 was reduced to amc. The author believed that reduction of ac_2 to amc in butter made with culture was promoted by the reducing action of the culture organisms. The loss of ac_2 in butter made without culture, and to a less extent in butter made with culture, was considered due to oxidation by air. Decomposition of ac_2 in oxidized and tallowy lots of butter was found to be less than might be expected;

this indicated that the fat peroxides may not be as important in the degradation of ac_2 as is generally supposed.

Virtanen and Tarnanen (129) stated that when deterioration in butter is caused by organisms (for example, bacteria of the *Ps. fluorescens* group) ac_2 is lost very quickly; this is evident before other defects become noticeable.

In the studies of Slatter (115) amc + ac_2 usually was not produced in salted butter. No conspicuous increases occurred, even when very little salt was present. Salt appeared to retard the decrease in amc + ac_2 in butter held at various temperatures.

Davies (23) reported that butter made from ripened cream must stand 16 to 24 hours before the maximum aroma develops. This phenomenon occurred at any temperature between 30° and 60° F. Very little increase in aroma occurred at 15° F. The increase in ac_2 after the manufacture of butter was considered to be due to bacterial action on amc and to autoxidation of amc. In cream after ripening there is no oxygen in solution but, after churning and working the butter, conditions are different in that the water in butter is saturated with atmospheric oxygen. The organisms in the buttermilk droplets in butter were believed to readjust the ac_2 : amc ratio to that existing in the early stages of cream ripening so that some of the excess amc is converted to ac_2. The high acidity of the buttermilk droplets was thought to cause a slight organic acidity, due largely to oleic acid being split from the fat. Free oleic acid in the presence of atmospheric oxygen was thought to develop small amounts of peroxides which oxidize some of the amc to ac_2. After 24 hours, the bacteria of the butter apparently utilize all the oxygen and there is no ac_2 formation. Davies concluded that the degree of flavor is lowered by a gradual oxidation of ac_2 to a flavorless product.

Barnicoat (7) observed no marked change in the ac_2 content of butter during frozen storage for 3 to 4 months. When butter was held at 40° F. for 7 to 10 days after manufacture, the ac_2 content decreased in some cases. Butter in which the greatest increases in ac_2 were observed were made from cream to which amc had been added and in which the culture was growing fairly vigorously at churning. Development of ac_2 in butter freshly made with culture was considered due to activity of the bacteria or their enzymes. The author concluded that the ac_2 content of butter made with culture, even after cold storage, generally is dependent on the concentration originally present in the cream. When ac_2 was added as the artificial substance, a decrease was observed; butter made with butter culture tended to increase slightly. Development of ac_2 in butter was not retarded, even when the butter was manufactured under reduced air pressure. An increase in ac_2 content of butter on holding 25 days at 40° F. was not considered due to atmospheric oxidation.

Bunger (16) found the ac_2 content of sour cream butter held at 62.6° to 71.6° F. remained practically the same during the first 4 days, whereas hold-

ing at 46.4° to 50° F. gave a slight increase in ac_2. After 12 days at 46.4° to 50° F., or higher, a decrease was noted in all cases. At 14° to 32° F. the ac_2 content was the same after 10 days.

According to Virtanen (128) the aroma of butter made from aerated cream, as well as that of butter made from non-aerated cream, greatly decreased during storage. Deterioration in butter made from aerated cream was no more extensive than in butter made from non-aerated cream; after 1 month both samples were graded approximately equal. Virtanen stated that disappearance of butter aroma during storage is an important problem for export countries, where butter cannot be sold fresh.

Matuszewski *et al.* (69) noted a distinct decrease and even disappearance of butter aroma in butter held 1 week at 50° to 59° F.; the butter decreased in both ac_2 and amc. Brioux and Jouis (13) reported that the amount of ac_2 in butter decreased rapidly. Within 15 to 18 days after manufacture, the ac_2 content of butter decreased to about one-tenth of its original value. One lot of butter containing 1.5 mg. ac_2 per kg. the day after churning contained only 0.05 mg. 26 days later.

Results of Davies (24, 25, 26) indicated that ripened cream butter requires from 12 to 24 hours before the full flavor develops. During this period amc is oxidized to ac_2 and an equilibrium is set up in the ratio of ac_2 to amc which is different from that in cream. Davies indicated that a different ratio is to be expected because there is practically no oxygen in solution in ripened cream while fresh butter is saturated with air.

Slatter and Hammer (117) did not find a significant increase in amc + ac_2 when salted butter was held at various temperatures. Butter containing 0.75 per cent salt showed a rather rapid disappearance of amc + ac_2 at 44°, 50° or 60° F. The amounts of amc + ac_2 in butter containing 1.0, 1.5 or 2.5 per cent salt remained fairly constant. Failure to obtain significant increases in the amc + ac_2 in salted butter was expected because of the restraining action of salt on the culture organisms.

Data obtained by Brioux and Jouis (14) indicated that in fresh butter ac_2 disappears rapidly when the butter is held. Butter containing 1.50 mg. ac_2 per kg. the day following manufacture, showed 0.60 mg. after 5 days, 0.13 mg. after 18 days and 0.05 mg. after 27 days. The amc content was 42.4 mg. per kg. the day following manufacture and 20.4 mg. after 27 days.

Pont (94) stated that full flavor does not develop in butter until approximately 24 hours after churning. There was no flavor development in butter held at the freezing point or lower.

Mohr *et al.* (78) found that sweet cream butter, with as much as 0.4 mg. ac_2 per kg. when fresh, contained practically none after 6 months in storage. When sour cream butter containing 1 to 2 mg. ac_2 per kg. was stored 6 months, the ac_2 content was reduced or remained constant. Various packing materials, such as parchment paper, transparent water-proof paper and

glass, had no effect on the ac_2 content. Storage of the butter in an atmosphere of carbon dioxide also had no effect. Iron and copper introduced into the butter from equipment increased the ac_2 content during storage but often caused off flavors.

Analyses on salted butter at various periods by Prill and Hammer (99) showed a rather high retention of ac_2 and amc, even at 70° F. Development of tallowiness at this temperature, which is common because of the great effect of relatively high temperatures on appearance of the defect, was not accompanied by a sharp decrease in ac_2 or amc contents.

Toth (125) noted that with butter prepared from acid cream the amount of aroma-producing substances increased for several days and then rapidly decreased. With butter made from sweet cream, a slow but constant decrease was observed; storage at low temperatures impeded these changes.

Hoecker and Hammer (47) obtained both increases and decreases in ac_2 with butter held 1 day at 40° F. and then 2 and 4 weeks at 0° or 35° F., the larger changes usually occurring at 35° F. Occasionally, increases in ac_2 contents after 2 weeks were followed by decreases after 4 weeks. Except in a few instances, the amc contents did not change appreciably.

Unsalted butter. Results obtained by Slatter (115) showed that amc + ac_2 commonly was produced in unsalted butter made with culture when the butter was held under favorable conditions, although the amounts in different lots held under the same conditions varied considerably. The largest production usually occurred at the highest holding temperature (60° F.) and in lots which developed the lowest pH. In several instances the amounts of amc + ac_2 in unsalted butter decreased during the first few days of holding and remained low, while in other cases a decrease was followed by an increase. A decrease regularly followed the maximum production of amc + ac_2. A larger production of amc + ac_2 occurred in the butter when 10, 15 or 20 per cent butter culture was added to the cream than when 5 per cent was used. In some cases addition of flavor-producing streptococci to the cream along with butter culture appeared to increase production of amc + ac_2 in butter during the holding.

Slatter and Hammer (117) found that when unsalted butter was held at 0°, 34° or 44° F. the amounts of amc + ac_2 sometimes increased and sometimes decreased, both increases and decreases being more definite at 44° F. than at lower temperatures. Increases commonly occurred at 50°, 60° or 70° F. At 50° and 60° F., maximum production usually was noted after 7 days. At 70° F. there was a decrease from the fourth to the seventh day. The pH of butter held at 44°, 50° and 60° F. was reduced gradually while at lower temperatures not much change occurred. A low pH in butter commonly was accompanied by a large production of amc + ac_2. Lowering of the pH and production of amc + ac_2 at relatively high temperatures sug-

gest that essentially the same changes take place in unsalted butter as in butter culture. The decrease in amc + ac_2 in unsalted butter is in agreement with the disappearance of these materials in butter cultures and is due, presumably, to reduction of amc or ac_2 to 2,3-butylene glycol by the flavor-producing streptococci. Development of amc + ac_2 at favorable temperatures suggests that a short ripening period may be desirable for commercial unsalted butter from the standpoint of obtaining flavor. However, these temperatures may also favor development of undesirable organisms.

Results of Mohr and Wellm (79) indicated that holding temperature exerts a decisive influence on the ac_2 and amc contents of butter. At 62.6°, 64.4° and 71.6° F. the amounts of ac_2 in sour cream butter remained practically constant for the first 4 days. Butter held at 50° F. usually showed an increase after 4 days. The ac_2 content decreased considerably in butter held 12 days above 46.4° F.; at 14° to 32° F. it remained practically constant, even after 12 days.

Prill and Hammer (99) found significant changes in ac_2 and in amc + ac_2 in unsalted butter held at 36° to 45° F. and at 70° F.; undoubtedly these were due to activity of the culture organisms. The changes involved both increases and decreases, as would be expected from the general relationship of the culture organisms to amc and ac_2. An increase in the ac_2 content of unsalted butter was believed to be important in the flavor development which this product often undergoes; a subsequent decrease presumably is accompanied by a partial loss of flavor.

Analyses by Hedrick and Hammer (45) on lots of unsalted butter before and after holding showed the following: Of 56 lots of butter held 1 week at 36° to 40° F., 47 showed increases in ac_2, 2 showed no change and 7 showed decreases; with the same lots, 38 showed increases in amc and 18 showed decreases. When the lots were held 3 days at 60° F. plus 4 days at 40° F., 45 showed increases in ac_2 and 11 showed decreases; with the same lots (minus 1), 37 showed increases in amc and 18 showed decreases. Of 43 lots of butter held 1 month at 36° to 40° F., 39 showed increases in ac_2 and 4 showed decreases; with the same lots (plus 1), 29 showed increases in amc, 1 showed no change and 14 showed decreases. The ac_2 and amc contents of butter held 3 days at 60° F. plus 4 days at 36° to 40° F. and of butter held 1 week at 36° to 40° F. were compared. The holding at 60° F. resulted in a higher ac_2 content in 42 of the 56 comparisons, no change in 1 and a decrease in 13; the amc content was higher in 40 of the 55 comparisons, the same in 4 and lower in 11.

ACTION OF ACETYLMETHYLCARBINOL AND DIACETYL ON BUTTER

Recognition of the importance of amc and ac_2 from the standpoint of flavor and flavor development in butter at once suggested consideration of the relationship of these compounds to butter deterioration. Two angles

are involved, chemical and microbiological. Since ac_2 is rather highly oxidized, it readily acts on certain compounds that are easily oxidized and in this way might produce off flavors in butter. Also, ac_2 has germicidal properties and might influence growth of microorganisms in butter, thus preventing development of certain off flavors.

Chemical Considerations

King (56) studied the influence of ac_2 on butterfat by adding it to melted and filtered fat, obtained from sweet cream, in concentrations of 0.05, 0.01 and 0.005 per cent. The fat used was almost odorless and the ac_2 went into it on warming. One series of tests was conducted in diffuse daylight at approximately 64.4° to 73.4° F. while the other was carried out in the dark at 71.6° to 75.2° F. In the presence of air the butterfat became bleached and tallowy. The change was accelerated by light. Bleaching was first evident at the surface and progressed from there. In general, tallowiness appeared later than the bleaching. Rate of bleaching and degree of tallowiness were proportional to the amount of ac_2 present. King suggested that ac_2 may act on butterfat by oxidizing oleic acid to oleic acid peroxide, which then breaks up into various compounds having a pronounced tallowy odor and flavor, the ac_2 being reduced to ame or even to 2,3-butylene glycol. The following reactions were suggested as explaining the action of ac_2 on oleic acid:

$$CH_3 \cdot (CH_2)_7 \cdot CH = CH \cdot (CH_2)_7 \cdot COOH$$

$$+ CH_3 \cdot CO \cdot CO \cdot CH_3 + \begin{matrix} H & O & H \\ H & O & H \end{matrix} + O_2 \rightarrow$$

$$CH_3 \cdot (CH_2)_7 \cdot \underset{\displaystyle O}{CH} - \underset{\displaystyle O}{CH} \cdot (CH_2)_7 \cdot COOH + CH_3 \cdot CO \cdot \overline{CHOH} \cdot \overline{CH_3} + H_2O_2$$

The hydrogen peroxide reacts with hydrogen to form water. Fixation of oxygen on the colored material of the fat also was suggested since the carotinoid pigments are rich in double bonds.

According to Tapernaux (120) the more aroma butter possesses, the poorer is its keeping qualities. Kay (54) reported that oxidation of ame during storage of butter indicates oxidative changes in the butterfat. Butter with pronounced aroma had poorer keeping qualities than butter with less aroma. Davies (22) noted that one of the disadvantages of ame in butter is its action as an oxidation catalyst, accelerating deterioration of the fat.

Hammer (40) stated that ac_2 is highly oxidized and can react with various materials. Large quantities of it bleached butterfat and produced tallowy odors and flavors. Small amounts, such as are present in butter, were not considered a direct cause of these defects. Action of ac_2 was consider-

ably slower if an acid, such as lactic or sulfuric, was present. Hammer noted that ac_2 worked into butter may have a better opportunity to react with fat and other constituents than if it is largely present in the moisture droplets of the butter. Later, in commenting on King's studies (56) Hammer (41) pointed out that butterfat exposed to air develops a tallowy odor and flavor, the action being surprisingly rapid. He also suggested that observations on the action of ac_2 on butterfat cannot be directly applied to the action of ac_2 when it is added to butter for the purpose of improving the flavor because of the very small amounts used and probably for other reasons also.

An article published by the Polaks Frutal Works (93), and intended to refute a published statement (2), indicated that ac_2 does not exert a definite oxidizing action on fats but has a tendency to reduce.

Barnicoat (5) did not consider ac_2 in itself to be responsible for the poorer keeping qualities of ripened cream butter. Later, he (7) stated that ac_2 has no effect on the keeping qualities of butter made from "mild starter" cream. Added ac_2 (6 ppm.) was not important in promoting deterioration of butter held 3.5 months at 14° F. Barnicoat noted that marked deterioration of high acid butter is related to low pH and not to its high ac_2 content.

Results obtained by Davies (23) led to the conclusion that with low-flavored butter it is unlikely the traces of ac_2 (0.1 to 0.3 ppm.) present will initiate autoxidation in the absence of considerable serum acidity; nor is this the case with medium-flavored butter (0.4 to 0.8 ppm. ac_2) or full-flavored butter (0.9 to 2.0 ppm. ac_2). Much larger concentrations of ac_2 (6.0 ppm.) were very active in promoting fat autoxidation, the compound being more active in this respect than an equivalent amount of oleic acid. Davies also reported (24) that tallowiness in full-flavored butter is not due so much to ac_2 as to conditions associated with manufacture of such butter. Later (25, 26, 27), he stated that full-flavored butter does not keep well but develops fishiness and oxidative rancidity much more rapidly than butter made from sweet or neutralized cream. The aroma constituent was not considered responsible for these defects but rather the high acid content. The amount of ac_2 in butter was regarded as too small to initiate or catalyze oxidative changes.

Pont (94) indicated that the peroxides formed as a result of the liberation of oleic acid oxidize ac_2 to flavorless compounds during butter deterioration. Further changes involve breakdown of lecithin, resulting in fishy flavor, and oxidation of butterfat, resulting in stale and tallowy flavors.

The peroxide numbers on samples of butterfat which were heated to 219.2° F. and held there for 8 hours were determined by Ritter and Nussbaumer (101). Addition of 0.1 or 1 per cent ac_2 to the butterfat resulted in a significant increase in peroxide number; the addition decreased the oxidation resistance of the butterfat. When butterfat contained 0.1 or 1

per cent added ac_2, it was much more susceptible to oxidation than control samples or samples containing less than 0.01 per cent ac_2. The greater the susceptibility of untreated butterfat to oxidation, the greater the accelerating influence of ac_2.

Wiley (136) stated that the presence of butter culture organisms in butter favored oxidation of the fat at cold storage temperatures. This was thought to be due to some product of bacterial metabolism acting as a prooxidant; the product was not amc or ac_2.

In referring to the work of King (56) Prill and Hammer (99) noted that he used pure butterfat with a relatively high concentration of added ac_2 and, in some cases, rather drastic treatments, such as exposure to light, while in butter it is probable that much of the ac_2 is in the water phase rather than entirely in the fat. In general, it appeared that the amount of ac_2 ordinarily encountered in butter has no significant effect on promotion of chemical defects and that other factors, such as copper content of the butter and pH of the serum, are of more importance in this connection.

Barnicoat (7) compared the vitamin A and carotene contents of lots of butter (made from portions of the same batch of cream) after 22 months at 14° to 17° F.; ac_2 was added to two of the lots during working at the rate of 4 ppm., or more than 12 times the concentration usually found in "mild starter" butter. The loss of vitamin A and its precursor carotene in both sweet cream and "mild starter" butter containing unusually large proportions of ac_2 was not greater than in control butter churned from sweet cream.

Hunziker and Cordes (48; p. 357) studied several thousand churnings of butter made from unripened cream in which ac_2 (approximately 3 to 4 ppm.) was added to the butter. After holding the butter 2 to 9 months, there was no indication of bleaching, tallowy flavors or other flavors such as might be attributable to oxidation by ac_2.

Toth (125) stated that too high a concentration of ac_2 is disadvantageous in the storing of butter since, on its reduction to amc, fats become rancid.

Microbiological Considerations

Lemoigne (61) stated that ac_2 in relatively small and variable amounts can retard development of micro-organisms. Experiments of Lévy-Bruhl and Cado (62) showed that *Staphylococcus aureus* was killed in bouillon by 0.1 per cent ac_2 but not by 0.04 per cent. Various organisms, including streptococci, pneumococci, gonococci and typhoid, paratyphoid, diphtheria and coliform types, were killed by 0.04 per cent but not by 0.02 per cent.

AMOUNTS OF ACETYLMETHYLCARBINOL AND DIACETYL IN VARIOUS DAIRY PRODUCTS

Over the years in which there has been an active interest in amc and ac_2 from the standpoint of flavor development in butter and related prod-

ucts, the methods of determination have changed greatly. For the detection of relatively small amounts of these compounds, colorimetric methods now are commonly used, instead of the less sensitive gravimetric methods (43; p. 156). This change in analytical procedure should be recognized in any consideration of the reported amc and ac_2 contents of dairy products.

Milk

Testoni and Ciusa (123, 124) did not find ac_2, amc or 2,3-butylene glycol in fresh milk; Mohler and Herzfeld (76) and also Pien *et al.* (91) did not detect ac_2 in it. Schmalfuss and Werner (112) reported that milk intended for household use contained no detectable amounts of ac_2 or amc, that is, less than 0.000024 per cent.

Ac_2 was not found in spontaneously soured milk by Testoni and Ciusa (123); later studies (124) indicated that old milk may contain some 2,3-butylene glycol. Schmalfuss and Barthmeyer (111) detected both ac_2 and amc in sour milk, about ten times more amc than ac_2 being present. Pien *et al.* (91) did not find ac_2 in acid coagulated milk.

Skimmilk

When 1 l. was used for the distillation, Krenn (58) did not find ac_2 in skimmilk. Schmalfuss and Werner (112) reported neither ac_2 nor amc in fresh skimmilk. Mohr and Wellm (79) believed that skimmilk contains both ac_2 and amc, although only to a very small extent (0.17 mg. ac_2 per l.).

Cream

Testoni and Ciusa (123) detected ac_2 in centrifuged cream. According to Michaelian and Hammer (73) sweet cream frequently contains a small amount of amc + ac_2; however, cream freshly skimmed from milk low in bacteria showed none in 200 g. Pien *et al.* (91) found no ac_2 in fresh cream. Slatter (116) stated that since a high acidity is necessary for production of ac_2, it is not found in sweet cream. Krenn (58) did not detect ac_2 in sweet cream when 1 l. was used for the distillation.

Tapernaux (120) reported that ac_2 can be detected in ripened cream, especially when the ripening is not preceded by pasteurization. According to Virtanen and Tarnanen (129) ripened cream contains practically no ac_2 but considerable amounts of amc. Pien *et al.* (91) obtained variable results when testing spontaneously soured cream for ac_2; the samples always showed some ac_2 (about 1.5 mg. per l.). Cream several weeks old showed about 1.3 mg. per l. Schmalfuss and Werner (112) found the ac_2 content of sour cream to be 0.000024 per cent. Slatter (116) stated that only small amounts of ac_2 are found in sour cream.

Additional data on the amc and ac_2 contents of cream intended for butter are given in the consideration of the influence of cream acidity on the amc and ac_2 contents of butter.

Other Products

Pien *et al.* (91) did not detect ac_2 in aqueous solutions of lactic acid from fermented milk. Considerable quantities of lactic acid gave a slight test for ac_2 and the distillate from 25 ml. syrupy lactic acid gave a positive reaction corresponding to 0.3 mg.

Schmalfuss and Werner (112) obtained a positive test for ac_2 on the material recovered by dry distillation of milk sugar. Dry distillation of citric acid did not give a positive test. The authors (112) found the ac_2 content of commercial margarine to be 0.00014 per cent.

Butter

van Niel *et al.* (126) found from 0.0002 to 0.0004 per cent ac_2 in fine butter. Schmalfuss and Barthmeyer (110) analyzed four samples of butter representing different conditions of feeding the producing animals and obtained the following weights of nickel salt (43; p. 156) equivalent to ac_2 per kg.: 0.0006, 0.0002, 0.0003 and 0.0001 g. The quantity of ac_2 in the butter appeared to be correlated with the intensity of the aroma. Testoni and Ciusa (122) stated that melted and pasteurized butter, as well as margarine, do not contain ac_2, whereas fresh butter contains about 0.00051 per cent. They believed the presence of ac_2 was a measure of the freshness of the butter. Later (123), they did not find ac_2 in properly prepared butter.

According to Tapernaux (120) fresh unpasteurized butter contains from 0 to 50 g. ac_2 in 100 kg.; pasteurized butter contained traces. Using a method capable of detecting 0.02 or more g. ac_2 per kg., Vizern and Guillot (130) obtained negative tests on butter. Hammer (40) found more amc than ac_2 in butter made with butter culture. Davies (22) stated that butter made from ripened cream may contain 0.05 to 0.5 ppm. of ac_2. Mohler and Herzfeld (76) reported that butter of normal aroma contains 0.0002 to 0.0004 per cent ac_2.

Michaelian and Hammer (73) examined 56 lots of butter for amc + ac_2. With a gravimetric method, 4 of them showed none while the remaining 52 yielded from 0.1 to 3.45 mg. nickel salt per 200 g. Steuart (118) found that the quantity of ac_2 in butter ranged from 0 to 5 ppm., depending on the presence and proportion of aroma bacteria in the souring cream. Testoni and Ciusa (124) stated that Italian butters do not normally contain ac_2; its presence was regarded as accidental. Pien *et al.* (91) found very little or no ac_2 in good butter (trace to 1 mg. per kg.).

Barnicoat (7) reported no correlation between the ac_2 content of butter and the score. Butter made with culture, which was quite acceptable to the graders, generally contained 0.2 to 0.4 ppm. ac_2 and 1 to 3 ppm. amc. "Mild starter" butter contained about eight times the ac_2 in sweet cream butter, but only one-fourth of that in highly flavored butter.

Of the butter samples analyzed by Brioux and Jouis (13), 76.3 per cent contained ac_2 in amounts ranging from a trace to 0.5 mg. per kg.; the re-

mainder contained from 0.51 to 1.5 mg., except in one instance for which the value was 2.5 mg. The amc contents were much higher, usually from 10 to 30 mg. per kg., with a maximum of 69 mg.

Davies (24) reported that the absence of ac_2 gives a flavorless butter, 0.2 to 0.6 ppm. gives a mild flavor and 0.7 to 1.5 ppm. gives a full flavor; 2 ppm. ac_2 gives a strong flavor and aroma which is harsh and repulsive to butter graders. Makar'in (67) stated that the aroma of butter is pronounced with an ac_2 content of 0.00048 per cent in the plasma. An ac_2 concentration as low as 0.0013 per cent produced an odd, sharp odor.

According to Matuszewski *et al.* (69) butter made from cream that had been inoculated with a culture of *S. diacetilactis* contained about 5 mg. nickel salt per kg.

Mohr and Wellm (79) noted that the method of producing butter, whether from sweet or sour cream, is of major importance with regard to the ac_2 content. Sweet cream butter contained considerably less ac_2 than sour cream butter. As much as 0.2 mg. ac_2 per kg. and 0.36 mg. amc + ac_2 was found in sweet cream butter. In various lots of German first-class butter, the ac_2 content varied from 0.34 to 1.66 mg. per kg. and the amc + ac_2 content from 3.73 to 20 mg.

Of the 130 samples of butter investigated by Pien *et al.* (92), 11 per cent contained ac_2 in amounts ranging from 0.5 to 1.0 mg. per kg., 47 per cent contained from 0.1 to 0.5 mg. and 42 per cent contained less than 0.1 mg.

Schmalfuss and Werner (112) found the ac_2 content of German butter made in August to be 0.00003 per cent. Slatter and Hammer (117) reported that the amount of amc + ac_2 in butter at the time of churning varied with the amount of butter culture used. Dehove and Dessirier (29) stated that all butter naturally contains a small amount of amc.

Brioux and Jouis (14) examined samples of fresh butter and found that they normally contained a small quantity of ac_2, usually from 0.05 to 0.5 mg. per kg.; in rare cases as much as 2.5 mg. was found. Butter made in cooperative or industrial creameries contained considerably more ac_2 than butter made on farms. The amc contents were considerably higher than the ac_2 contents; usually butter contained between 10 and 30 mg. per kg. but some lots contained as much as 69 mg. Amc did not appear to give rise to an appreciable quantity of ac_2.

Alberti (1) found the amount of ac_2 in butter varied from a trace to 0.4 mg. per kg. when the samples were from the same source. The amc contents were from 10 to 40 times the ac_2 contents.

According to Mohr *et al.* (78) fresh sweet cream butter contained up to 0.4 mg. ac_2 per kg. and sour cream butter from 1 to 2 mg. Salted and unsalted butter showed no difference in ac_2 contents.

In comparisons involving addition of equal percentages of regular and aerated or modified aerated (p. 88; p. 89) culture to pasteurized sweet cream,

Prill and Hammer (98) noted that butter made with the latter types of culture invariably had the higher ac_2 contents (0.38 to 1.0 mg. per kg. compared to 0.14 to 0.38 mg.). However, the higher values were much lower than they would have been if the ac_2 contents of the butter were proportional to those of the cultures.

Toth (125) found that Hungarian butter contained 0.05 to 1.5 mg. per cent ac_2 and 1.5 to 4.2 mg. per cent amc.

Hoecker and Hammer (47) noted that butter made without butter culture or with *S. aromaticus* contained only small amounts of ac_2 and amc, whereas butter made with butter culture, *S. citrovorus, S. paracitrovorus, S. diacetilactis, S. citrophilus* or an unidentified organism commonly contained appreciable amounts.

BUTTER CULTURE DISTILLATE AS A SOURCE OF FLAVOR FOR BUTTER

Amc in various materials is readily converted to ac_2 by oxidation with some such reagent as ferric chloride, and the ac_2 thus formed, as well as that originally present, can be distilled out. Distillates obtained from butter culture with this general procedure (often called starter distillates) have been suggested for producing flavor in butter, the distillates being worked into the butter during the normal working process. Because a butter culture contains much more amc than ac_2, a distillate contains much more ac_2 than the volume of culture from which it was obtained. However, some of the amc distills over before its conversion to ac_2 (72) and appears as such in the distillate.

Since volatile acids, and possibly other volatile compounds, contribute to the flavor of butter culture, a culture distillate may contain materials other than ac_2 that are desirable from the standpoint of butter flavor. On the basis of the present knowledge, volatile acids appear to be of most importance in this connection.

Ruehe and Ramsey (107) noted that the flavor constituents of butter cultures, including ac_2 and amc, are removed by steam distillation. Addition of the distillate to sweet cream before churning yielded butter of intensified aroma. Similar results were obtained by adding the distillate directly to butter. Butter cultures used for steam distillation were grown in such a manner that they contained increased amounts of the flavoring constituents.

Use of an ac_2 concentrate was suggested by Davies (23). The concentrate was prepared by distillation of ac_2 from butter culture and addition of the distillate to neutralized butter culture. Such preparations contained 300 times more ac_2 than ripened cream.

According to Ruehe (105) the ac_2 content of butter culture distillate does not change appreciably, even after 63 days at 40° F. When butter was made with various amounts of distillate, most judges preferred that containing less than 1 part ac_2 in 200,000 parts of butter.

The scheme suggested by Ruehe (106) for controlling flavor in butter consists of selection of cultures which actively produce lactic acid and ferment citrates when grown in milk, adoption of methods which are conducive to high yields of amc, steam distillation of the culture, standardization of the ac_2 content of the distillate and addition of the distillate to the churned butter at the time of salting. Advantages of the suggested method are that the distillate can be prepared in the laboratory and supplied to various plants, flavor intensity can be adjusted to demands of the trade, high-flavored butter with good keeping qualities can be produced, butter has a low bacterial count and culture distillate is more economical than butter culture. In a series of comparisons involving use of 2.5 per cent butter culture and various amounts of ac_2 as butter culture distillate, the butter containing ac_2 as distillate in the ratio of 1 part ac_2 to 400,000 parts of butter scored highest when fresh; after 2 months at 40° F., the butter containing ac_2 as distillate in the ratio of 1 part ac_2 to 800,000 parts of butter scored highest. Similar results were obtained when the lots of butter were held at −10° F. for 8 months and scored at intervals of 2 months.

DETECTION OF ADDED DIACETYL IN BUTTER

In the examination of butter, detection of ac_2 added as such, or in the form of a flavor concentrate, sometimes is attempted. It is complicated by the fact that ac_2 commonly is present in butter through use of butter culture and/or cream containing ac_2.

Davies (22) noted that a relatively high ac_2 content and a low acidity in butter should indicate that ac_2 had been added artificially. Later, he (27) reported that analytical evidence for detection of added synthetic ac_2 in butter is unsatisfactory. The two main evidences of such addition are acidity of the butter serum and ratio of ac_2 to amc. The author stated that butter serum having a pH of 6.4 to 6.8 should not contain a detectable amount of ac_2. The ratio of ac_2 to amc in normal butter was considered to be from 1 : 15 to 1 : 20. Butter culture distillates had ratios of 5 : 1 to 2 : 1.

The ratio of ac_2 to amc in butter was suggested by Barnicoat (5) as of value in the detection of added ac_2. Bacteriological examination was proposed as a guide for determining whether butter culture had been used in the cream from which the butter was churned.

Pien *et al.* (92) stated that since none of the butter examined contained more than 1.0 mg. ac_2 per kg., samples containing larger amounts had ac_2 added to them artificially.

Hoecker and Hammer (47) studied pure milk cultures of various streptococci, including *S. citrovorus, S. paracitrovorus, S. diacetilactis, S. citrophilus,* an unidentified organism and *S. aromaticus.* With each species the ratio of ac_2 to amc varied in the different trials; frequently the ac_2 was much higher in proportion to the amc than with butter cultures. The results

suggest the dangers in using the ac_2 to amc ratio in detecting added ac_2 in butter.

REFERENCES

(1) ALBERTI, CARLO. Ann. Chim. Appl., 28: 475–482. 1938.
(2) ANONYMOUS. Dairy World (London) Dec. 16, 1933. (Quoted by Polaks Frutal Works; Natl. Butter and Cheese Jour., 25: 22. 1934.)
(3) ANONYMOUS. Jour. Dept. Agr., Union S. Africa, 8: 551. 1924; Chem. Abs., 18: 3436. 1924. Orig. not seen.
(4) ASCHAN, OSSIAN. Finska Kemistsamfundets Medd., 38: 6–18. 1929; Chem. Abs., 24: 437. 1930. Orig. not seen.
(5) BARNICOAT, C. R. Analyst, 60: 653–662. 1935.
(6) BARNICOAT, C. R. Jour. Dairy Res., 6: 397–406. 1935.
(7) BARNICOAT, C. R. Jour. Dairy Res., 8: 15–30. 1937.
(8) BOGDANOW, W. M. Milch. Butterindustrie, 4: 5–9. 1937 (Russisch); Abs. Milchw. Forsch., 19: 208. 1938. Orig. not seen.
(9) BOGDANOW, W. M. Moloch-Maslodel'naya Prom., 4: 5–9. 1937; Chem. Abs., 33: 7411. 1939. Orig. not seen.
(10) BOUSKA, F. W. Amer. Creamery and Poultry Prod. Rev., 81: 365–366. 1936.
(11) BREWER, C. R., MICHAELIAN, M. B., WERKMAN, C. H., AND HAMMER, B. W. Jour. Bact., 33: 92. 1937.
(12) BREWER, C. R., WERKMAN, C. H., MICHAELIAN, M. B., AND HAMMER, B. W. Iowa Agr. Expt. Sta., Res. Bul., No. 233. 1938.
(13) BRIOUX, CHR., AND JOUIS, EDG. Compt. Rend. Acad. Sci. (Paris), 205: 526–528. 1937; Ann. falsif., 30: 457–469. 1937.
(14) BRIOUX, CHR., AND JOUIS, EDG. Lait., 18: 11–22. 1938.
(15) BROWN, C. W., SMITH, L. M., AND RUEHLE, G. L. A. JOUR. DAIRY SCI., 3: 375–405. 1920.
(16) BUNGER, H. Landw. Jahrb. Schweiz., 84: 887–927. 1937.
(17) CLAYDON, T. J., AND HAMMER, B. W. Iowa Agr. Expt. Sta., Res. Bul., No. 267. 1939.
(18) CONN, H. W. Conn. (Storrs) Agr. Expt. Sta., 2nd Ann. Rep., 52–67. 1889.
(19) CONN, H. W. Conn. (Storrs) Agr. Expt. Sta., 3rd Ann. Rep., 136–157. 1890.
(20) CONN, H. W. Conn. (Storrs) Agr. Expt. Sta., 6th Ann. Rep., 43–68. 1893.
(21) CROSSLEY, E. L. Soc. Chem. Indus. Jour., 57: 319–321. 1938.
(22) DAVIES, W. L. Food Mfr., 8: 346–348. 1933.
(23) DAVIES, W. L. Dairy Indus., 1: 165–167. 1936.
(24) DAVIES, W. L. XI. Milchwirtschaftlicher Weltkongress, Berlin, 2: 76–78. 1937.
(25) DAVIES, W. L. Agr. Prog., 15: 30–34. 1938.
(26) DAVIES, W. L. Flavours, 1: 27–34. 1938.
(27) DAVIES, W. L. Flavours, 2: 19–23, 32. 1939.
(28) DEAN, H. H. Ontario Agr. Col. Expt. Farm., 30th Ann. Rep., 73–88. 1904.
(29) DEHOVE, R., AND DESSIRIER, L. Lait., 18: 150–153. 1938.
(30) DERBY, H. A., AND HAMMER, B. W. Iowa Agr. Expt. Sta., Res. Bul., No. 145. 1931.
(31) FABRICIUS, N. E. Dairy Indus., 5: 159–160. 1940.
(32) FABRICIUS, N. E. The Creamery Jour., 52: No. 7, 5, 28–30. 1941.
(33) FABRICIUS, N. E., AND HAMMER, B. W. Iowa Agr. Expt. Sta., Res. Bul., No. 221. 1937.
(34) FERRIS, L. W., REDFIELD, H. W., AND NORTH, W. R. JOUR. DAIRY SCI., 4: 521–535. 1921.

(35) FOUTS, E. L. JOUR. DAIRY SCI., 23: 303–306. 1940.
(36) GARRISON, E. R., AND HAMMER, B. W. Iowa State Col. Jour. Sci., 16: 363–377. 1942.
(37) GRIMES, M. JOUR. DAIRY SCI., 6: 427–445. 1923.
(38) HAMMER, B. W. Iowa Agr. Expt. Sta., Res. Bul., No. 67. 1921.
(39) HAMMER, B. W. Proc. World's Dairy Congr., 2: 985–993. 1923.
(40) HAMMER, B. W. Natl. Butter and Cheese Jour., 24: No. 12, 28–30. 1933.
(41) HAMMER, B. W. Proc. 7th Ann. State Col. Wash. Inst. Dairying 1934, pp. 16–20.
(42) HAMMER, B. W. Dairy Indus., 3: 407–408, 412. 1938.
(43) HAMMER, B. W., AND BABEL, F. J. JOUR. DAIRY SCI., 26: 83–168. 1943.
(44) HAMMER, B. W., AND JENSEN, C. Natl. Butter and Cheese Jour., 22: No. 9, 12–15. 1931.
(45) HEDRICK, T. I., AND HAMMER, B. W. Iowa Agr. Expt. Sta., Res. Bul., No. 301. 1942.
(46) HOECKER, W. H., AND HAMMER, B. W. Iowa Agr. Expt. Sta., Res. Bul., No. 290. 1941.
(47) HOECKER, W. H., AND HAMMER, B. W. JOUR. DAIRY SCI., 25: 175–185. 1942.
(48) HUNZIKER, OTTO FREDERICK. The Butter Industry. 3rd Ed. Published by the author. LaGrange, Ill. 1940.
(49) HUSSONG, R. V., LONG, H. F., AND HAMMER, B. W. Iowa Agr. Expt. Sta., Res. Bul., No. 225. 1937.
(50) HUSSONG, R. V., QUAM, SIDNEY, AND HAMMER, B. W. JOUR. DAIRY SCI. (Accepted for publication.)
(51) ITZEROTT, A. G. F. Jour. Dept. Agr., Victoria, 39: 39–42. 1941.
(52) JACKSON, H. C. N. Y. (Cornell) Agr. Expt. Sta., Memoir 71. 1923.
(53) JACOBSEN, D. H., AND EVANS, T. ALLEN. South Dakota Agr. Expt. Sta., Res. Bul., No. 350. 1941.
(54) KAY, H. D. Soc. Chem. Ind. Jour., 52: 624–630. 1933.
(55) KHUBCHANDANI, PRITO G. Agr. Live-Stock India, 9: 162–166. 1939; Chem. Abs., 34: 1760. 1940. Orig. not seen.
(56) KING, N. Milchw. Forsch., 12: 172–182. 1931.
(57) KNUDSEN, SØNCKE. Jour. Dairy Res., 2: 137–163. 1931.
(58) KRENN, JOSEF. XI. Milchwirtschaftlicher Weltkongress, Berlin, 2: 78–80. 1937.
(59) LANGTON, H. M. Flavours, 2: 17–23. 1939.
(60) LEITCH, M. Lait., 4: 732–739. 1924.
(61) LEMOIGNE, M. Compt. Rend. Soc. Biol., 97: 1479–1481. 1927.
(62) LÉVY-BRUHL, M., AND CADO, YVONNE. Compt. Rend. Soc. Biol., 122: 373–374. 1936.
(63) LIND, C. Lait., 7: 538–544. 1927.
(64) LONG, H. F., AND HAMMER, B. W. Iowa Agr. Expt. Sta., Res. Bul., No. 263. 1939.
(65) LUCAS, P. S., BALL, C. D., VINCENT, R. E., AND TROUT, G. M. Mich. Agr. Expt. Sta., Quart. Bul., 14: 38–41. 1931.
(66) MADDOCK, E. C. G. Jour. Dairy Res., 2: 203–207. 1931.
(67) MAKAR'IN, A. M. Moloch-Maslodel'naya Prom., 4: 20–22. 1937; Milchw. Forsch., 19: 208. 1938; Chem. Abs., 33: 9467. 1939. Orig. not seen.
(68) MAKAR'IN, A. M. Moloch-Maslodel'naya Prom., 5: 10–13. 1938; Chem. Abs., 35: 2988. 1941. Orig. not seen.
(69) MATUSZEWSKI, T., PIJANOWSKI, E., AND SUPINSKA, J. XI. Milchwirtschaftlicher Weltkongress, Berlin, 2: 80–85. 1937.
(70) MAZÉ, M. Compt. Rend., 143: 1198–1201. 1906.
(71) MAZÉ, M. XI. Milchwirtschaftlicher Weltkongress, Berlin, 2: 85–89. 1937.

(72) MICHAELIAN, MICHAEL B. B. W. Hammer Panegyric. Collegiate Press, Inc., Ames, Iowa, pp. 223–240. 1937.
(73) MICHAELIAN, M. B., AND HAMMER, B. W. Iowa Agr. Expt. Sta., Res. Bul., No. 179. 1935.
(74) Minn. Agr. Expt. Sta., Ann. Rep., 1919. pp. 44–45.
(75) MINSTER, J. T. Analyst, 57: 615–621. 1932.
(76) MOHLER, H., AND HERZFELD, E. Mitt. Lebensmtl. Hyg., 26: 34–41. 1935.
(77) MOHR, W., AND ARBES, A. Fette u. Seifen, 46: 214–217. 1939.
(78) MOHR, W., SCHRIMPL, E., AND ARBES, A. Molk.-Ztg., 53: 1501–1503. 1939; Milchwirtschaftlicher Literaturbericht, p. 423, 1939; Chem. Abs., 34: 535. 1940. Orig. not seen.
(79) MOHR, W., AND WELLM, J. XI. Milchwirtschaftlicher Weltkongress, Berlin, 2: 89–97. 1937.
(80) MORTENSEN, M. Iowa Agr. Expt. Sta., Bul. No. 207. 1922.
(81) NELSON, J. A., AND HAMMER, B. W. JOUR. DAIRY SCI., 16: 375–385. 1933.
(82) NEUBERGER, L. Chemiker-Ztg., 22: 217–218. 1939; Chem. Zentralbl., 1939, II, S. 3644–3645. Orig. not seen.
(83) Ontario Agr. Col. Expt. Farm, Ann. Rep., 48: 29–36. 1922.
(84) ORLA-JENSEN, S. Zentr. Bakt., Parasitenk., II., 8: 11–16, 42–46, 74–80, 107–114, 140–144, 171–174, 211–216, 248–252, 309–312, 342–346, 367–369, 406–409. 1902.
(85) ORLA-JENSEN, S. Dairy Bacteriology. P. Blakiston's Son and Co., Inc., Philadelphia. 2nd English Ed. 1931.
(86) OVERMAN, O. R. Natl. Butter and Cheese Jour., 27: No. 12, 6–7. 1936.
(87) OVERMAN, O. R., GARRET, O. F., AND RUEHE, H. A. Ill. Agr. Expt. Sta. Bul. 446. 1938.
(88) PARFITT, E. H. World's Butter Review, 1: No. 3, 11–15. 1927.
(89) PARSONS, C. H. Amer. Creamery and Poultry Prod. Rev., 81: 364–365. 1936.
(90) PIEN, JEAN. Ann. falsif., 30: 473–484. 1937.
(91) PIEN, J., BAISSE, J., AND MARTIN, R. Lait., 16: 119–138, 243–255. 1936.
(92) PIEN, J., BAISSE, J., AND MARTIN, R. Lait., 17: 673–698. 1937.
(93) POLAKS FRUTAL WORKS. Natl. Butter and Cheese Jour., 25: No. 3, 22. 1934.
(94) PONT, E. G. Jour. Australian Inst. Agr. Sci., 4: 131–136. 1938.
(95) PONT, E. G. Jour. Council Sci. Indus. Res., 14: 1–10. 1941.
(96) PRICE, F. E., HURD, C. J., AND WILSTER, G. H. Oregon Agr. Expt. Sta., Bul., No. 305. 1932.
(97) PRILL, E. A., FABRICIUS, N. E., AND HAMMER, B. W. Iowa Agr. Expt. Sta., Res. Bul., No. 268. 1939.
(98) PRILL, E. A., AND HAMMER, B. W. JOUR. DAIRY SCI., 22: 79–88. 1939.
(99) PRILL, E. A., AND HAMMER, B. W. JOUR. DAIRY SCI., 23: 159–168. 1940.
(100) RAHN, OTTO, BROWN, C. W., AND SMITH, L. M. Mich. Agr. Expt. Sta., Tech. Bul., No. 2. 1909.
(101) RITTER, W., AND NUSSBAUMER, TH. Schweiz. Milch Ztg., 65: 193. 1939.
(102) ROGERS, LORE A. U. S. Dept. Agr., Bureau Animal Indus., Bul. No. 57. 1904.
(103) ROGERS, LORE A., Associates of. Fundamentals of Dairy Science. Reinhold Publishing Corp., 330 W. 42nd St., New York. 2nd Ed. 1935.
(104) ROGERS, L. A., AND GRAY, C. E. U. S. Dept. Agr., Bureau Animal Indus., Bul. No. 114. 1909.
(105) RUEHE, H. A. Natl. Butter and Cheese Jour., 28: No. 2, 20–22. 1937.
(106) RUEHE, H. A. The Creamery Jour., 49: 5, 28. 1938.
(107) RUEHE, H. A., AND RAMSEY, R. J. Milk Plant Monthly, 24: No. 2, 40. 1935.
(108) RUEHLE, G. L. A. Mich. Agr. Expt. Sta., Quart. Bul., 6: 24–25. 1923.

(109) SAYER, WILLIAM S., RAHN, OTTO, AND FARRAND, BELL. Mich. Agr. Expt. Sta., Tech. Bul., No. 1. 1909.
(110) SCHMALFUSS, HANS, AND BARTHMEYER, HELENE. Biochem. Ztschr., 216: 330–335. 1929.
(111) SCHMALFUSS, HANS, AND BARTHMEYER, HELENE. Ztschr. Untersuch. Lebensmtl., 63: 283–288. 1932.
(112) SCHMALFUSS, H., AND WERNER, H. Fette u. Seifen., 44: 509–514. 1937.
(113) SHERWOOD, F. F., AND HAMMER, B. W. Iowa Agr. Expt. Sta., Res. Bul., No. 90. 1926.
(114) SHUTT, D. B. Sci. Agr., 9: 316–320. 1929.
(115) SLATTER, WALTER L. Natl. Butter and Cheese Jour., 27: No. 20, 20–24; No. 21, 18–26. 1936.
(116) SLATTER, WALTER L. Natl. Butter and Cheese Jour., 28: No. 17, 38–39. 1937.
(117) SLATTER, W. L., AND HAMMER, B. W. Iowa Agr. Expt. Sta., Res. Bul., No. 211. 1937.
(118) STEUART, D. W. Analyst, 60: 172–173. 1935.
(119) ST. VON NYIREDY. XI. Milchwirtschaftlicher Weltkongress, Berlin, 2: 137–140. 1937.
(120) TAPERNAUX, A. Volume Jubilaire en L'Honneur Du Professeur Charles Porcher-Chambéry, Imprimeries Réunies, 3, Rue Lamartine, 1932; Lait., 12: 1043–1055. 1932.
(121) TEMPLETON, HUGH L. Wis. Agr. Expt. Sta., Res. Bul., No. 133. 1937.
(122) TESTONI, G., AND CIUSA, W. Ann. Chim. Appl., 21: 147–150. 1931.
(123) TESTONI, G., AND CIUSA, W. Ann. Chim. Appl., 22: 44–48. 1932.
(124) TESTONI, GUISEPPE, AND CIUSA, WALTER. Ann. Chim. Appl., 25: 39–41. 1935.
(125) TOTH, JENÖ. Mezögazdasági Kutatások, 14: 47–65. 1941; Chem. Abs., 35: 4862. 1941. Orig. not seen.
(126) VAN NIEL, C. B., KLUYVER, A. J., AND DERX, H. G. Biochem. Ztschr., 210: 234–251. 1929.
(127) VIRTANEN, A. I. 9th Intern. Dairy Congr. Report of the Delegation of the U. S. to the Sec. of State, p. 42. 1931.
(128) VIRTANEN, ARTTURI I. XI. Milchwirtschaftlicher Weltkongress, Berlin, 2: 121–123. 1937.
(129) VIRTANEN, ARTTURI I., AND TARNANEN, J. Acta. Chem. Fenn., 9B: 2. 1936.
(130) VIZERN AND GUILLOT. Ann. falsif., 25: 459–462. 1932.
(131) WALTS, C. C. Ark. Agr. Expt. Sta., Bul. No. 249. 1930.
(132) WASER, E., AND MOHLER, H. Mitt. Lebensmtl. Hyg., 23: 265–267. 1932.
(133) WASHBURN, R. M., AND DAHLBERG, A. C. JOUR. DAIRY SCI., 1: 114–126. 1917.
(134) WHITE, WILLIAM, TRIMBLE, C. S., AND WILSON, H. L. U. S. Dept. Agr., Tech. Bul., No. 159. 1929.
(135) WILEY, W. J. Jour. Council Sci. Indus. Res., 10: 327–332. 1937.
(136) WILEY, W. J. Jour. Dairy Res., 10: 300–309. 1939.
(137) WILEY, W. J., COX, G. A., AND WHITEHEAD, H. R. New Zealand Dept. Sci. Indus. Res., Ann. Rep., 13: 20. 1939; Chem Abs., 34: 1760. 1940. Orig. not seen.
(138) WILSTER, G. H. B. W. Hammer Panegyric. Collegiate Press, Inc., Ames, Iowa, pp. 75–82. 1937.

A SATISFACTORY METHOD OF SHIPPING DAIRY BULL SEMEN LONG DISTANCES*

ERIC W. SWANSON AND H. A. HERMAN
Department of Dairy Husbandry, University of Missouri, Columbia, Mo.

The methods of collecting, storing, and handling bull semen after collection have been widely investigated in recent years for the purpose of extending the use of valuable sires through artificial insemination (1, 3, 5, 6). Satisfactory progress has been made in this field especially in its application to large cooperative associations where the bulls are within easy reach of the cows to be inseminated. It is often desirable, however, to breed certain cows to bulls which are a great distance away. Shipment by the ordinary means of transportation available may require two days or more in many instances. This situation presents a different transportation and storage problem than is encountered in shorter shipping distances.

Numerous investigators (1, 2, 3, 5, 6) have shown that the proper temperature for storage of bull semen is below 45° F. and preferably at or slightly below 40° F. or 5° C. Hence, some reliable practical method of refrigeration and temperature control is necessary. Glass vacuum bottles alone may be used, but in addition to being very fragile, they are unsuited for lengthy shipment because of the relatively short period at which a temperature as low as 40° F. may be maintained. A light cardboard shipping container, described by Salisbury (4) has proved suitable in artificial breeding association work where the shipping time does not exceed 24 hours. Ice-filled thermal jugs likewise have been used successfully for short-time shipment. In addition to providing only a short safe shipping time, these types of packages have given difficulty because the semen is in close contact with ice which in cold weather may cause the temperature of the semen to drop too low. An investigation was therefore conducted to find a shipping method that could be depended upon to maintain proper refrigeration for 48 hours or more and at the same time not harm the semen by too low temperatures. A prime consideration was to adopt a container which would withstand the rigors of shipping. The type of package finally adopted and preliminary results from its use are reported below.

The requirements of long distance shipping were; 1) adequate refrigeration of the package, 2) protection of the semen from the intense cold of the refrigerant, and 3) insulation of the refrigerant against the external temperature. It was desired to meet these requirements as simply as possible so that the method would be quickly and easily available to all who wished

Received for publication July 6, 1943.

* Contribution by the Department of Dairy Husbandry, Missouri Agricultural Experiment Station, Journal Series No. 906.

to use it. For this reason utilization was made of existing equipment entirely. The refrigerant used was mainly crushed ice with a can of frozen brine used for extra hot weather. A small vacuum bottle filled with water at 40° F. was used to contain the vials of semen; and commercially available small insulated shipping packages such as are commonly used for ice cream were used as external insulation. These were combined as diagrammed in figure 1.

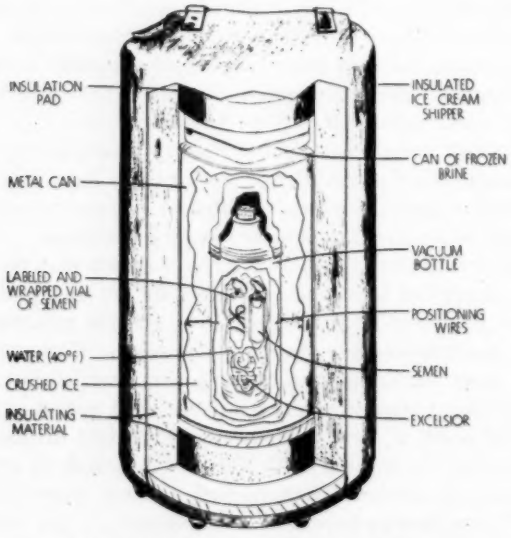


FIG. 1. A cut-away diagram of the packed semen shipper.

The cooled or partially cooled semen in an insulated, rubber-wrapped vial was placed in a vacuum bottle of water at 40° F. A few strands of excelsior in the bottle prevent undue bobbing of the vials during transit. The vacuum bottle, with supporting wires to position it at the center, was put into a metal can and chipped ice was packed around it as shown in figure 1. The can was sealed and placed in a small ice cream shipper. Best results were secured when the can fit in the shipper tightly or was packed in with insulating material. If extra refrigeration is desired a can of frozen brine may be placed on top of the can. The shipper should be packed quickly, closed, and shipped. The packed shipper weighs between 15 and 20 pounds, depending upon how much ice is used; so it can be easily shipped by express or parcel post.

This shipper has maintained a satisfactory semen storage temperature for more than 84 hours at summer temperatures (80° F.) and several ship-

ments (two in hot August weather) have been successfully made in which the package was three days in transit. Maximum holding time can be obtained by precooling the shipper and using a maximum of ice plus the frozen brine pad.

The semen is protected from the severe cold of the ice by means of the water in the vacuum bottle. The size of the vacuum bottle and the amount of ice used can be varied for different outside temperature conditions. In summer shipments a one-half pint vacuum bottle provided enough water insulation so that the temperature of the semen did not fall below 34° F. Also, a large amount of ice could be packed around this small bottle. In cold winter weather, however, only a small amount of ice was needed in the can to insure proper refrigeration. A full can of ice around a one-half pint bottle was not satisfactory with the outside temperature below 32° F. because the temperature of the semen dropped to 32° F. Three different shipments of semen known to be fertile under proper storage conditions produced no pregnancies when they had been cooled to 32° F. By using a pint bottle in place of the one-half pint bottle and packing the can only half full with crushed ice, the temperature of the water did not fall below 34° F. and the fertility of the shipped semen was maintained very satisfactorily. Therefore, the recommended procedure is to use a pint vacuum bottle and a small amount of ice in winter conditions and to use a one-half pint bottle with a maximum amount of ice in hot summer conditions.

The glass vacuum bottles in these containers are subject to shattering under very rough handling. With ordinary care and handling, however, no breakage has occurred. If breakage of the bottle does occur, the chances are still good for the semen to come through in satisfactory condition because of the refrigerant being outside of the bottle.

Twenty-four shipments of semen have been made by this method in the course of twelve months' investigations. When the proper precautions as outlined above have been observed, the shipments have been successful in delivering semen with motility as good or better than that obtained from the same semen stored at 40° F. in a refrigerator. Furthermore, the few inseminations made have yielded satisfactory results. Eight conceptions have been secured from twelve inseminations using semen ranging from 48 to 130 hours of age regardless of its motility rating. The semen used ranged from 70 to 90 per cent motility when packed and was from University of Missouri bulls known to have a low insemination-per-conception ratio. The inseminations made with the semen which was cooled to 32° F. in transit were not included in this summary. Six inseminations which were made with semen which had been stored 144 hours or more (all of very poor motility) were infertile. Results have been, therefore, such as would be expected from the use of properly handled stored semen. The success in shipping semen by the method described will depend largely upon the ability of the semen to maintain fertility during storage at 35 to 45° F.

SUMMARY

A simple, dependable, and practical method of shipping semen long distances is described. The package consists of a small insulated ice cream shipper, a water-tight metal can to fit the shipper, crushed ice, and a vacuum bottle. Satisfactory semen storage temperatures (35 to 50° F.) were maintained in this shipper for 84 hours at atmospheric temperature of 80° F. Results of inseminations with shipped semen requiring 48 to 72 hours in transit were very satisfactory for stored semen.

REFERENCES

(1) Davis, H. P., Underbjerg, G. K. L., and Williams, N. K. The Effect of Storage Temperatures upon Certain Characteristics of Bovine Semen. Jour. Dairy Sci., 23: 1057–1068. 1940.

(2) Easley, G. T., Mayer, D. T., and Bogart, R. Influence of Diluters, Rate of Cooling, and Storage Temperatures on Survival of Bull Sperm. Amer. Jour. Vet. Res., 3: 358–363. 1942.

(3) Lambert, W. V., and McKenzie, F. F. Artificial Insemination in Livestock Breeding. U. S. D. A. Cir. 567. 1940.

(4) Salisbury, G. W. Storing, Packaging, and Shipping Semen. Jour. Dairy Sci., 25: 673. 1942.

(5) Swanson, E. W., and Herman, H. A. Storage of Dairy Bull Spermatozoa. Jour. Dairy Sci., 24: 579–587. 1941.

(6) Willet, E. L., and Salisbury, G. W. The Effect of Various Diluters, Cooling Ratè, Temperature of Storage, and Some Other Factors on the Livability of Spermatozoa in Stored Samples of Bull Semen. Cornell Univ. Agr. Expt. Sta. Mem. 249. 1942.

THE ACCURACY OF THE MOJONNIER METHOD FOR ESTIMATING MILK FAT IN MILK AND CREAM

ERNEST O. HERREID AND DANA W. WHITMAN[1]
Vermont Agricultural Experiment Station[2]

Laboratory technicians have inquired about the degree of accuracy that can be achieved in estimating milk fat in milk by the Mojonnier method. They have also questioned the relative accuracy of single or duplicate determinations on individual samples. Data are presented for milk and cream to answer these questions.

LITERATURE REVIEW

The literature related to this study is the result of comparing the accuracy of the various volumetric methods with the Röese-Gottlieb method and its mechanized modification, the Mojonnier method, for estimating milk fat in milk and cream. The literature is cited if the data are complete and sufficient to make a contribution of statistical value.

Phillips (4) estimated 50 samples of unpreserved milk in duplicate for milk fat. The individuals in 29 duplicates agreed, 15 differed by 0.01 per cent, 5 by 0.02 per cent and 1 by 0.03 per cent milk fat. Dahlberg, Holm and Troy (1) made 55 estimations for milk fat in duplicate. Some of the estimations were made on replicate samples in different laboratories by the Röese-Gottlieb and the Mojonnier methods. Of the 55 estimations, 14 agreed, 8 differed by 0.01, 18 by 0.02, 9 by 0.03, 1 by 0.04, 2 by 0.05, 1 by 0.07, 1 by 0.22 and 1 by 0.29 per cent milk fat. Fisher and Walts (2) made 32 estimations and approximately 81 per cent of the individuals in duplicates did not differ by more than 0.04 per cent milk fat.

METHODS

The methods of preparing the samples and estimating them for milk fat have been described (3). After preparing the samples of milk and cream, they were transferred directly to the extraction flasks and weighed (3). Four technicians participated in this study.

It was necessary to obtain some information on the accuracy of estimating milk fat in replicate samples. One hundred estimations were made on 100 samples of the same milk that was produced during the month of June. These individual samples were prepared by agitating 40 quarts of fresh, raw milk at 8–10° C. for about two minutes. As the milk was being

Received for publication July 9, 1943.

[1] The assistance of Charles Harmon and Harriet Mueller is gratefully acknowledged.

[2] Published with the approval of the Director of the Vermont Agricultural Experiment Station.

gently agitated, approximately 100 ml. were poured with a dipper into each of 102 sterile sample jars; two extra samples were taken in case some of them might be accidentally broken. The bottles, closed with rubber stoppers, were held at 10° C. or lower before being estimated for milk fat. The estimations were made in groups of eight samples insofar as possible. About 36 hours were required in the laboratory to make the 100 determinations. The last samples were about 52 hours old when all the estimations were completed. The titratable acidity in the fresh, cold milk at 9–10° C. was 0.13 per cent calculated as lactic acid and was 0.16 per cent under comparable conditions after 52 hours. The bacterial count of the fresh milk at the beginning of the trial was 12,800 per ml.

RESULTS

The estimations for milk fat on the 100 replicate samples are given in Table 1 and are averaged in groups of 25. It is evident that there is a gradual and slight consistent decrease in the amount of milk fat from the first to the last group. In fact, the last group averaged about 0.03 per cent lower than the first group of 25. It is not known what caused this gradual

TABLE 1

The estimations for milk fat on 100 replicate samples of unpreserved milk

Sample number	Per cent milk fat (1)	Sample number	Per cent milk fat (2)	Sample number	Per cent milk fat (3)	Sample number	Per cent milk fat (4)
1	3.71	26	3.76	51	3.72	76	3.70
2	3.74	27	3.70	52	3.68	77	3.71
3	3.74	28	3.71	53	3.72	78	3.71
4	3.75	29	3.73	54	3.73	79	3.75
5	3.74	30	3.72	55	3.72	80	3.73
6	3.72	31	3.70	56	3.72	81	3.68
7	3.72	32	3.73	57	3.73	82	3.68
8	3.74	33	3.73	58	3.72	83	3.67
9	3.74	34	3.74	59	3.71	84	3.66
10	3.75	35	3.71	60	3.71	85	3.68
11	3.73	36	3.71	61	3.71	86	3.67
12	3.75	37	3.71	62	3.73	87	3.72
13	3.70	38	3.73	63	3.71	88	3.66
14	3.74	39	3.71	64	3.72	89	3.73
15	3.70	40	3.73	65	3.72	90	3.69
16	3.73	41	3.73	66	3.71	91	3.72
17	3.74	42	3.72	67	3.72	92	3.69
18	3.76	43	3.71	68	3.72	93	3.71
19	3.73	44	3.73	69	3.71	94	3.73
20	3.72	45	3.73	70	3.72	95	3.72
21	3.73	46	3.73	71	3.72	96	3.71
22	3.74	47	3.71	72	3.72	97	3.68
23	3.71	48	3.73	73	3.74	98	3.70
24	3.73	49	3.73	74	3.72	99	3.69
25	3.72	50	3.72	75	3.72	100	3.73
Mean	3.73	.....	3.72	.....	3.72	.....	3.70
Mean of all	.....	.....	.....	.....	.....	.....	3.72

decrease in milk fat when the samples are held for longer periods, but an observation will be mentioned. Samples 81–88, inclusive, were prepared and weighed into the extraction flasks at the end of the second day. Due to the late hour, the flasks containing the milk were allowed to remain in the laboratory over night and the ether extractions were completed the next day. No sour odors could be detected from these 8 samples the next morning. These samples (81–88) averaged 3.68 per cent milk fat, which is significantly lower than the average for the 100 samples. The other 17 samples in the same group averaged 3.71 per cent milk fat.

The frequency distribution in table 2 shows that 82 of the estimations vary from the mean by 0.02 per cent or less and 18 by more than 0.02 per cent milk fat. Therefore, the odds are 82 to 18 or 4.56 to 1 that individual

TABLE 2

Frequency distribution of estimations for milk fat on 100 replicate samples of unpreserved milk

Per cent milk fat	Frequency
3.66	2
3.67	2
3.68	5
3.69	3
3.70	6
3.71	19
3.72	24
3.73	23
3.74	10
3.75	4
3.76	2
	100

Mean = 3.72 per cent.
Standard deviation = 0.02 per cent.

estimations will not vary from the mean by more than about 0.02 per cent and will agree within 0.04 per cent milk fat. Furthermore, 76 per cent of the estimations are in the frequency groups of 3.71 to 3.74, a difference of 0.03 per cent milk fat.

The results in table 2 should serve as a basis to predict the accuracy that may be attained in routine analyses. To test the validity of this hypothesis, the differences between individuals for each of 341 duplicate estimations on fresh milk were calculated. These estimations were made at regular intervals over a period of about two years. The results are given in the third column of table 3. It is evident that nearly 77 per cent of the individuals in duplicates agreed within 0.03 per cent milk fat and approximately 86 per cent of them within 0.04 per cent milk fat; this agrees closely with the results in table 2.

To show the comparative accuracy of single and of duplicate estimations, it is assumed that the average of duplicates will give the most accurate

results. The variation of the individuals from their mean is one-half of the difference between single determinations for each sample of milk. These calculations were made on the same 341 samples of milk and the results are given in the fourth column of table 3. Eighty-six per cent of the individuals in duplicates did not vary from their means by more than 0.02 per cent milk fat, about 94 per cent by not more than 0.03 per cent milk fat and about 98 per cent by not more than 0.04 per cent milk fat.

Bimonthly composite milk samples preserved with bichloride of mercury were estimated for milk fat in duplicate. These samples were obtained from two milk plants during the summer months. Sixty samples were obtained

TABLE 3

Frequency distribution of differences between the individuals in duplicates and between individuals and the means of their duplicates for milk fat estimations on 341 samples of unpreserved milk

Frequency	Per cent	Differences between individuals	
		Duplicates	Means of duplicates
		Per cent milk fat	
70	20.53	0.00	0.000
94	27.57	0.01	0.005
63	18.48	0.02	0.010
36	10.56	0.03	0.015
32	9.38	0.04	0.020
16	4.69	0.05	0.025
12	3.52	0.06	0.030
6	1.76	0.07	0.035
6	1.76	0.08	0.040
2	0.59	0.09	0.045
1	0.29	0.10	0.050
1	0.29	0.11	0.055
1	0.29	0.12	0.060
1	0.29	0.13	0.065
341	100.00		

from Plant A. These samples were in excellent physical condition because they had been properly refrigerated and cared for in the milk plant during the 15-day period. Another set of sixty samples was obtained from Plant B. These samples were not in good physical condition. Some of them showed varying degrees of fat destabilization and mold growth. The results in table 4 show that 95 per cent of the individuals in duplicate estimations from Plant A agreed within 0.03 per cent milk fat and all of them within 0.05 per cent; furthermore, the differences between individual estimations and the means of their duplicates on all the samples are less than 0.03 per cent milk fat. As might be expected, the variability of the results was greater from Plant B, about 73 per cent of the individuals agreeing within 0.03 per cent milk fat and 85 per cent within 0.04 per cent. The increased variability of the results from Plant B can be attributed to the difficulty of obtaining a

TABLE 4

Frequency distribution of differences between individuals in duplicate estimations for milk fat on 120 samples of preserved milk

Differences milk fat per cent	Plant			
	A		B	
	Frequency	Per cent	Frequency	Per cent
0.00	13	21.67	6	10.00
0.01	19	31.67	19	31.67
0.02	19	31.67	8	13.33
0.03	6	10.00	11	18.33
0.04	.....	.....	7	11.67
0.05	3	5.00	2	3.33
0.06	.....	.....	2	3.33
0.07	.....	.....	.....	.....
0.08	.....	.....	3	5.00
0.09	.....	.....	2	3.33
	60	100.01	60	99.99

representative sample, because of the destabilized condition of the fat emulsion. The technician who made the estimations on the preserved samples was able to achieve greater accuracy than any of the other three who participated in this work.

Two hundred and forty-two samples of unpreserved cream containing 20–45 per cent of milk fat were estimated for milk fat in duplicate at regular intervals over a period of about two years. It is evident from table 5 that approximately 86 per cent of the individuals in duplicate estimations agreed within about 0.30 per cent milk fat. Approximately 86 per cent of the individuals in the duplicates did not vary more than 0.15 per cent milk fat and about 97 per cent not more than 0.30 per cent from their means.

TABLE 5

Frequency distribution of differences between the individuals in duplicates and between individuals and the means of their duplicates for milk fat estimations on 242 samples of unpreserved cream

Frequency	Per cent	Differences between individuals	
		Duplicates	Means of duplicates
		Per cent milk fat	
103	42.56	0.0–0.09	0.0 –0.045
68	28.10	0.1–0.19	0.05–0.095
38	15.70	0.2–0.29	0.10–0.145
8	3.31	0.3–0.39	0.15–0.195
12	4.96	0.4–0.49	0.20–0.245
7	2.89	0.5–0.59	0.25–0.295
3	1.24	0.6–0.69	0.30–0.345
1	0.41	0.7–0.79	0.35–0.395
1	0.41	0.8–0.89	0.40–0.445
1	0.41	0.9–0.99	0.45–0.495
242	99.99		

DISCUSSION

An accuracy can be achieved with single estimations on normal milk samples that may not vary more than 0.03 per cent milk fat in at least 75 per cent of the cases and 0.04 per cent milk fat in at least 82 per cent of the cases (tables 2, 3). An accuracy can be achieved with duplicate estimations that may not vary more than 0.02 per cent milk fat in about 86 per cent of the cases, 0.03 per cent milk fat in about 94 per cent of the cases, and 0.04 per cent milk fat in about 98 per cent of the cases (tables 3, 4).

For cream, approximately 86 per cent of the individuals in duplicate estimations did not vary more than 0.29 per cent milk fat, and about 86 per cent of the individuals in duplicates did not vary more than 0.145 per cent milk fat from their means.

The comparative accuracy of the Mojonnier method on milk and cream can be calculated. Assuming that cream contains 40 per cent milk fat, then the variation between individuals in 86 per cent of the duplicate estimations would be 0.725 per cent of the total milk fat $(0.29 \div 0.40 \times 100 = 0.725)$. Using this same method of calculating and assuming that milk contains 4 per cent of milk fat, then the variation between individuals in at least 82 per cent of the cases would be one per cent of the total milk fat $(0.04 \div 4 \times 100 = 1)$. Therefore, a slightly higher degree of accuracy can be achieved on cream than on milk.

The condition of the fat emulsion affects the degree of accuracy that can be achieved. Approximately 73 per cent of the individuals in duplicates agreed to 0.03 per cent milk fat from Plant B (table 4) as compared to 95 per cent of the individuals from Plant A. The fat emulsion in the samples from Plant B showed varying degrees of destabilization. Duplicate estimations should be made on milk samples if the fat emulsion is abnormal. For commercial and routine analyses, single estimations on individual samples of normal milk may give sufficient accuracy for practical purposes; however, when a high degree of accuracy is desired, duplicate estimations should be made. The importance of the results to be achieved should determine whether single or duplicate estimations for milk fat are to be made on samples of milk and cream.

CONCLUSIONS

1. Single estimations of the milk fat content of milk by the Mojonnier method can be expected to give an accuracy within 0.03 and 0.04 per cent milk fat in at least 75 and 82 per cent, respectively, of the cases.

2. The mean of duplicate estimations can be expected to give an accuracy within 0.02 and 0.03 per cent milk fat in approximately 86 and 94 per cent, respectively, of the cases for normal milk.

3. Single estimations of the milk fat content of normal cream by the Mojonnier method can be expected to give an accuracy of about 0.30 per cent milk fat in about 86 per cent of the cases.

4. Duplicate estimations for normal cream can be expected to give an accuracy within 0.30 per cent milk fat in about 97 per cent of the cases.

REFERENCES

(1) DAHLBERG, A. C., HOLM, G. E., AND TROY, H. C. A Comparison of the Babcock, Gerber and Röese-Gottlieb Methods for Determining the Percentage of Fat in Milk and Cream. N. Y. Agr. Expt. Sta. Tech. Bul. 122. 1926.

(2) FISHER, R. C., AND WALTS, C. C. A Comparative Study of Methods of Determining the Percent of Fat in Dairy Products Conn. Agr. Expt. Sta. Bul. 131. 1925.

(3) HERREID, E. O., AND HARMON, C. A Study of Methods of Obtaining Milk Samples for Estimating Milk Fat by the Mojonnier Method. JOUR. DAIRY SCI., 27: 33–38. 1944.

(4) PHILLIPS, C. A. A Comparison of the Babcock and the Röese-Gottlieb (Mojonnier) Methods for Determination of Butter Fat in Milk. JOUR. DAIRY SCI., 6: 549–555. 1923.

THE MILK AND BUTTERFAT PRODUCTION RESPONSES TO SHARK LIVER OIL IN THE RATION*

F. C. FOUNTAINE AND D. W. BOLIN

Departments of Dairy Husbandry and Agricultural Chemistry, University of Idaho, Moscow

Reports on the results of feeding shark liver oil to dairy cattle are conflicting.

Deuel and co-workers (1, 4) reported that the daily feeding of 30 to 60 cc. of shark liver oil increased the milk and butterfat production of Guernsey cows approximately 10 per cent. This amount of shark liver oil furnished from 700,000 to 1,400,000 I.U. of Vitamin A.

Rupel, Boyer and Phillips (8) found that the daily feeding of 25 cc. of shark liver oil with a Vitamin A potency of 7,500 I.U. and 15,000 I.U. per gram had no effect on the milk production and fat percentage of Holstein and Guernsey cows. These authors reported that the daily feeding of 90 cc. of a 15,000 I.U. shark liver oil caused a noticeable drop in the amount of 4 per cent fat-corrected milk.

More recently Jensen *et al.* (5) reported that daily feeding of shark liver oil to furnish from 117,500 to 1,350,000 I.U. of Vitamin A resulted in no increase in milk or butterfat production of Jersey, Guernsey, Holstein, and Brown Swiss cows. It was found that the daily feeding of as much as 90 cc. of shark liver oil tended to increase the normal rate of decline in milk production with the advance of lactation.

In view of these conflicting reports the results presented in this paper are offered as additional information.

EXPERIMENTAL

Twelve cows, 6 Jerseys and 6 Holsteins, were used in the experiment. These cows were divided into 4 groups; group I, Holstein supplement, group II Jersey supplement, group III, Holstein control and group IV, Jersey control. The groups were so divided that the production of each was approximately that of its control group.

All groups were fed the same basal ration, consisting of alfalfa hay, grain mixture and sunflower silage, which was replaced by sweet clover silage after 8 weeks. The grain mixture consisted of barley, oats, wheat bran, linseed oil meal, cottonseed meal, dried molasses beet pulp, iodized salt, and steamed bone meal.

All groups were fed the basal ration during a preliminary period of 4 weeks. Beginning with the fifth week the rations of groups I and II were

Received for publication July 13, 1943.

* Approved for publication by the Director of the Idaho Agricultural Experiment Station as Research Paper No. 222.

supplemented with 60 cc. daily of shark liver oil with a Vitamin A potency of 25,000 I.U. per gram.

The duration of the experiment was 16 weeks, beginning on January 3, 1942, and ending April 30, 1942.

The shark liver oil supplement was discontinued April 18, 1942, but the cows were kept under observation until April 30, 1942.

Daily milk weight records were kept on all cows during the preliminary and the oil feeding periods. Composite milk samples were taken daily from each cow and the per cent butterfat determined by the Babcock method. Once a week an additional composite milk sample was taken from each cow for Vitamin A and carotene determination.

The butterfat was extracted from the milk and treated prior to digestion by the method of Olson *et al.* (7). Vitamin A and carotene determinations on the unsaponified residue were made according to the method of Koehn and Sherman (6).

RESULTS

The daily feeding of 60 cc. of shark liver oil with a Vitamin A potency of 25,000 I.U. per gram had no effect on milk or butterfat production. These data are presented in figure 1 and table 1.

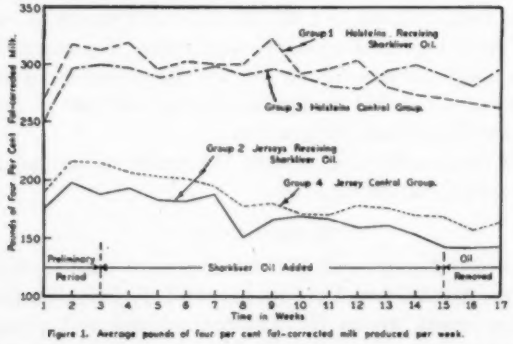


Figure 1. Average pounds of four per cent fat-corrected milk produced per week.

FIG. 1. Average pounds of four per cent fat-corrected milk produced per week.

The data in table 1 show that the feeding of shark liver oil caused a marked increase in the Vitamin A content of the butterfat. This increase was approximately 280 per cent for the Holstein cows and 305 per cent for the Jersey cows. The vitamin A content of the butterfat reached its greatest value the second week after the cows received the supplement. After this the Vitamin A content of the butterfat dropped somewhat, but was maintained at a considerably higher level than that of the cows receiving no supplement.

The carotene content of the butterfat of the cows receiving the supplement gradually decreased during the course of the experiment (table 1).

TABLE 1

The effect of shark liver oil feeding on the milk and fat production and the vitamin A and carotene content of the milk fat of Holstein and Jersey cows

Period week ending	Group I Holstein Basal ration + 60 cc. shark liver oil			Group III Holstein Basal ration		
	Av. weekly milk yield, lbs. 4% F.C.M.	Av. vitamin A, I.U./gm. milk fat	Av. carotene, micrograms/gm. milk fat	Av. weekly milk yield, lbs. 4% F.C.M.	Av. vitamin A, I.U./gm. milk fat	Av. carotene, micrograms/gm. milk fat
Preliminary period						
1– 9–42	269.7	.....	.....	248.1	.....	.....
1–16–42	318.7	24.2	4.4	297.0	33.2	3.9
1–23–42	312.6	18.6	3.6	299.8	16.9	2.6
1–30–42	319.6	20.3	3.0	293.6	19.8	2.4
Experimental period						
2– 6–42	297.6	52.6	3.2	289.0	20.5	2.0
2–13–42	303.2	69.0	3.6	293.3	23.7	2.9
2–20–42	301.5	44.3	3.0	299.5	22.0	2.9
2–27–42	301.4	57.7	2.9	292.4	20.7	2.8
3– 6–42	291.6	54.4	2.9	297.8	20.5	2.8
3–13–42	293.9	66.5	2.2	290.2	21.3	2.4
3–20–42	287.7	64.9	3.2	282.0	20.1	2.7
3–27–42	304.7	53.0	2.4	279.3	20.3	2.9
4– 3–42	280.7	57.9	2.4	294.6	20.9	2.5
4–10–42	274.0	45.4	1.8	299.8	16.3	3.4
4–17–42	271.4	52.8	1.9	296.3	15.7	2.4
4–24–42*	266.2	55.0	2.7	280.5	21.2	3.6
5– 1–42	262.3	42.1	3.6	297.2	31.0	4.3

	Group II Jersey			Group IV Jersey		
Preliminary period						
1– 9–42	175.5	.....	.....	190.0	.....	.....
1–16–42	198.3	18.7	6.2	216.7	20.5	6.0
1–23–42	187.5	15.3	5.3	214.0	16.6	4.1
1–30–42	193.1	12.4	4.2	206.3	12.6	3.3
Experimental period						
2– 6–42	184.2	43.7	4.5	204.1	24.2	3.9
2–13–42	181.7	71.5	4.5	202.9	29.3	4.3
2–20–42	225.1	39.2	3.9	195.7	18.6	4.2
2–27–42	181.3	48.1	3.9	177.9	23.5	4.5
3– 6–42	166.2	44.3	3.2	180.7	23.8	3.9
3–13–42	168.8	53.4	3.0	171.2	24.7	3.6
3–20–42	166.1	49.3	3.6	170.9	24.9	3.7
3–27–42	159.2	39.4	2.8	178.1	28.9	3.8
4– 3–42	161.8	53.6	3.5	176.9	25.2	4.3
4–10–42	153.2	39.8	2.9	170.2	22.8	4.3
4–17–42	142.9	42.3	3.1	165.9	22.9	3.8
4–24–42*	141.8	51.4	2.9	190.6	24.7	4.9
5– 1–42	142.1	29.8	4.6	164.8	25.9	6.1

* Shark liver oil removed from ration.

There was an average reduction of 22 per cent in carotene content of the Holstein butterfat, and 33 per cent in the carotene content of the Jersey butterfat.

DISCUSSION

The data presented show that the daily administration of 60 cc. of shark liver oil furnishing a daily intake of 1,350,000 I.U. of Vitamin A had no effect on milk or butterfat production. This supports the results of Jensen *et al.* (5) and is contrary to the findings of Deuel and co-workers (1, 4).

The increase in the Vitamin A content of the milk is in agreement with the results of Deuel and co-workers (2, 4) and Jensen *et al.* (5).

While the increase in Vitamin A content of milk fat was not as marked as that reported by Deuel, it was significant. The decrease in carotene content of the butterfat supports the results of Deuel (3) and of Jensen *et al.* (5).

SUMMARY

1. The daily feeding of 60 cc. of shark liver oil furnishing 1,350,000 I.U. of Vitamin A had no effect on milk or butterfat production of either Holstein-Friesian or Jersey cows.

2. The daily feeding of 60 cc. of shark liver oil resulted in a 280 to 305 per cent increase in the Vitamin A content of the milk fat.

3. The feeding of shark liver oil caused a 22 per cent average decrease in the carotene content of the Holstein milk fat, and a 33 per cent average decrease in the carotene content of the Jersey milk.

REFERENCES

(1) Deuel, H. J., Jr., Halliday, N., Hallman, L. F., and Johnston, C. The Effect of Vitamin A Intake on Vitamin A Content of Butter Fat. Jour. Biol. Chem. 139: 479. 1941.

(2) Deuel, H. J., Jr., Halliday, N., Hallman, L. F., Johnston, C., and Miller, A. J. The Production of High Vitamin A Milk by Diet. Jour. Nutr. 22: 303. 1942.

(3) Deuel, H. J., Jr., Hallman, Lois F., Johnston, C., and Mattson, J. The Effect of High Vitamin A Intake on the Blood and Milk Carotene of Holstein and Guernsey Cows. Jour. Nutr. 23: 567. 1942.

(4) Deuel, H. J., Jr., and Nuthall, J. P. The Effect of Shark Liver Oil on the Vitamin A Content of Milk and on Milk Production. Certified Milk, 16: 3. 1941.

(5) Jensen, C., Boyer, P. D., Phillips, P. H., Rupel, I. W., and Lundquist, N. S. The Effect of Shark Liver Oil on Milk and Butter Fat Production. Jour. Dairy Sci., 25: 931. 1942.

(6) Koehn, C. J., and Sherman, W. C. The Determination of Vitamin A and Carotene with the Photoelectric Colorimeter. Jour. Biol. Chem., 132: 527. 1940.

(7) Olson, F. R., Hegsted, D. M., and Peterson, W. H. Determination of Carotene and Vitamin A in Milk. Jour. Dairy Sci., 12: 63. 1939.

(8) Rupel, I. W., Boyer, Paul D., and Phillips, Paul H. The Effect of Shark Liver Oil Feeding upon Milk Production and Blood Vitamin A. Jour. Animal Sci., 1: 68. 1942.

A 2×2 FACTORIAL DESIGN FOR DOUBLE REVERSAL FEEDING EXPERIMENTS

D. M. SEATH[1]
Louisiana Agricultural Experiment Station

Investigators in the dairy field have for years made liberal use of the double-reversal design for conducting feeding trials. This design employs the procedure of using two groups of cows (A and B) and starting period I with the A group on ration X and the B group on ration Y. At the end of each period the rations are reversed; thus, in period III the groups are fed the same rations as in period I. In the analysis the production during period II is compared to the average for periods I and III. The comparison thus made is considered appropriate because of the usual decline in production of cows as the lactation progresses. The assumption is made that if other factors remain under control the cows will produce as much in period II as the average of periods I and III.

One of the strongest criticisms against employing the double reversal design is that there is a residual, or carry-over effect, caused by the previous rations fed. In 1941 Cochran, Autrey and Cannon (1), in a discussion of a double change-over design presented a method for correcting this influence. A partial correction for this carry-over effect is also made possible by discarding the records for a preliminary period at the beginning of each feeding period.

A method for determining the significance of the differences found in the reversal or switch-back trials was presented by Brandt in 1938 (2). An extension of this test is herein presented so as to permit the experimenter to compare two sets of factors at the same time—such as two concentrates and two roughages—with an appropriate method of testing the significance of the differences found between each set of factors. The design where applicable can employ the same number of cows to answer two questions as is commonly used to answer a single question. Caution should be exercised in its use, however. The experimenter should know from previous knowledge or experience that the differences between the two roughages is likely to be of about the same magnitude while on each of the two concentrate mixtures.

THE EXPERIMENTAL PLAN

The particular design presented grew out of an attempt to use a limited number of cows in two feeding trials to compare two kinds of hay and two concentrate mixtures. Rather than conduct separate trials it was decided

Received for publication July 20, 1943.

[1] The author is greatly indebted to Dr. W. G. Cochran of the Statistical Laboratory of Iowa State College for guidance in developing the analysis as herein presented.

to combine the trials with the major group, A or B, getting the same kind of hay (alyce or lespedeza)[2] and the subgroups, a and b, differing in the kind of concentrate, either three parts corn and one part cottonseed meal or three parts dehydrated sweet potato meal and one part cottonseed meal. Twenty

TABLE 1

Alyce clover vs. lespedeza and corn vs. dehydrated sweet potato meal in the dairy ration (Production of 4% equivalent milk)

Hay group	Grain group	Cow No.	Period I	Period II	Period III	$P_1 - 2P_2 + P_3$
			P_1	P_2	P_3	
			Alyce + s. pot.	Lesp. + corn	Alyce + s. pot.	
A	a	1	369.7	337.6	303.9	− 1.6
		2	376.1	380.8	413.8	+ 28.3
		3	440.0	376.0	407.4	+ 95.4
		4	375.0	362.2	344.5	− 4.9
		5	248.5	257.5	278.5	+ 12.0
		Sums	1809.3	1714.1	1748.1	+129.2
			Alyce + corn	Lesp. + s. pot.	Alyce + corn	
	b	1	206.4	196.1	210.6	+ 24.8
		2	486.0	423.4	469.7	+108.9
		3	425.0	350.7	373.6	+ 97.2
		4	342.5	302.9	362.9	+ 99.6
		5	474.9	422.5	442.3	+ 72.2
		Sums	1934.8	1695.6	1859.1	+402.7
			Lesp. + s. pot.	Alyce + corn	Lesp. + s. pot.	
B	a	1	182.1	207.7	199.7	− 33.6
		2	436.1	501.0	430.5	−135.4
		3	341.3	378.0	338.18	− 76.6
		4	373.9	427.8	374.3	−107.4
		5	383.1	404.6	345.8	− 80.3
		Sums	1716.5	1919.1	1688.4	−433.3
			Lesp. + corn	Alyce + s. pot.	Lesp. + corn	
	b	1	257.1	253.3	239.5	− 10.0
		2	436.7	461.3	487.9	+ 2.0
		3	489.3	484.3	441.0	− 38.3
		4	308.4	332.3	307.8	− 48.4
		5	235.5	245.0	248.6	− 5.9
		Sums	1727.0	1776.2	1724.8	−100.6

cows were used in the trial with 10 in each hay group and these were subdivided into two five-cow concentrate groups. Table 1 presents the plan

[2] The lespedeza hay used was approximately one grade lower in quality than was the alyce clover hay. The comparisons made are for the purposes of presenting a method of analysis rather than to give experimental results.

along with the resulting milk yields for the cows for each of the three periods. Each period consisted of 21 days; 7 days as a preliminary period and 14 days as a test period.

THE ANALYSIS

1. *An Example Showing Significant Differences*

The present experiment conforms to a factorial pattern, an experimental design described in detail by Yates (4). This particular factorial design involves variations in two sets of factors, so is designated as a 2×2 factorial, and has the interaction between the two sets of factors confounded with cows. Our notation on the factors involved is:

First factor	*Second factor*
1 Alyce clover	1 Sweet potato
2 Lespedeza	2 Corn

Using this notation the sub-group totals in table 1 are:

$$A(a)\ 11-22=+129.2$$
$$A(b)\ 12-21=+402.7$$
$$B(a)\ 21-12=-433.3$$
$$B(b)\ 22-11=-100.6$$

The sub-total differences are derived by adding sums for Periods I and III and subtracting twice the sum for Period II. Thus, $11-22$, or the subgroup difference in production between alyce + sweet potatoes and lespedeza + corn equals $1809.3-2(1714.1)+1748.1$ or $+129.2$. A study of the subgroup totals immediately gives evidence that alyce clover + corn was the best combination, for this ration as shown in B(a) excelled lespedeza and sweet potatoes by 433.3 pounds of milk. This type of comparison, however, involves possible first-order interactions which are confounded with cows in this experiment. These interactions are ignored and the first problem at hand is to determine the differences between the two kinds of hay and the two concentrate mixtures. They are:

$$\text{alyce} - \text{lespedeza} = 129.2 + 402.7 - (-433.3) - (-100.6) \text{ or } 1065.8$$
$$\text{S. pot.} - \text{corn} = 129.2 + (-433.3) - 402.7 - (-100.6) \text{ or } -606.2$$

These differences show in one case the superiority of alyce clover over lespedeza and in the other the inferiority of sweet potato meal as compared to corn. The next procedure is to determine the significance of these differences.

The Analysis of Variance employing the calculation of the sum of squares for a single degree of freedom as described by Snedecor (3), will first be used. In terms of a single difference $(P_1 - 2P_2 + P_3)$ the sum of squares for these components are:

$$\frac{(1065.8)^2}{20^*} = 56{,}796 \qquad \frac{(-606.2)^2}{20} = 18{,}373$$

* The denominator equals nk or the number of major groups (2) × the number of cows in each group (10) or 20. See (3).

The individual cow differences as well as the sub-group totals under the heading $P_1 - 2P_2 + P_3$ in table 1 are employed in computing the error sum of squares, thus: $(-1.6)^2 + (28.3)^2 + (95.4)^2 \ldots + (5.9)^2 - 1/5\ [(129.2)^2 + (402.7)^2 + (-433.3)^2 + (-100.6)^2] = 26{,}703$ with 16 degrees of freedom. This gives for the analysis of variance:

Source of variance	*Degrees of freedom*	*Sum of squares*	*Mean square*	*F*
Alyce vs. lespedeza	1	56,796	56,796	34**
S. pot. vs. corn	1	18,373	18,373	11**
Error	16†	26,703	1,669	.....

The analysis shows that both sets of factors were significantly different at the one per cent level**; with alyce clover excelling lespedeza, and corn excelling sweet potato meal.

An analysis involving the use of group means and testing the significance by the use of the standard error will next be presented. Table 2 shows a 2×2 table with totals. The treatment means per cow per period with differences, and the respective standard errors of these differences are also given.

TABLE 2

Milk yields by treatments—with means and differences

	Alyce	Lespedeza	Mean	Difference
S. potatoes	355.5	339.8	347.6	15.2 ± 4.567
Corn	381.6	344.0	362.8	
Mean	368.6	341.9		
Difference	26.7 ± 4.567			

Calculating these means on a per cow per period basis envolves using sub-group totals from table 1; thus, for alyce + s. pot. $= 1809.3 + (2 \times 1776.2) + 1748.1/20$ or 355.5. In like manner the other three totals in table 2 are calculated. From this the variance of a figure in this 2×2 table becomes:

$$5/400\ [1^2 + 2^2 + 1^2] \times (\text{variance per cow per period}) \doteq$$

$$3/40\ \sigma^2, \text{ where } \sigma^2 = \frac{1{,}669‡}{6}. \quad \text{This gives } 20.86.$$

The standard error of the difference $= \sqrt{20.86}$ or 4.567

$$t = \frac{\text{Mean difference}}{\text{S.E.}} = \frac{26.7}{4.567} = 5.85^{**}$$

Likewise

$$\frac{15.2}{4.567} = 3.33^{**}$$

The values of t, as was the case with F in the analysis of variance, are significant at the one per cent point. The t and F tests are, of course, identical.

† The error term has 16 degrees of freedom, four from each group.

‡ The method for obtaining 1669 is given for Error Variance in the analysis of variance and 6 is the sum of exponents $1^2 + 2^2 + 1^2$.

2. *An Example Showing non-Significant Differences*

Liveweight changes as well as milk and butterfat yields are used as "measuring sticks" in double reversal feeding trials. Their use in the experiment just described furnishes data to illustrate a case where the differences observed failed to be significant at the five per cent level. The use of the analysis of variance to test this significance will be presented.

Table 3 presents the liveweight changes for each cow and the sums for the sub-groups. The effects of the rations are shown by

Alyce-lespedeza = (−64 − 434) − (+33) − (−118) or −413
S. pot.-corn = (−64 + 33) − (−434) − (−118) or 521

TABLE 3

Summary of liveweight changes

Hay group	Grain group	Cow No.	Period I	Period II	Period III	$P_1 - 2P_2 + P_3$
			P_1	P_2	P_3	
			Alyce + s. pot.	Lesp. + corn	Alyce + s. pot.	
A	a	1	+38	+ 18	−25	− 23
		2	+10	− 15	+42	+ 82
		3	− 3	+ 45	−10	−103
		4	+ 9	+ 10	−12	− 23
		5	−14	+ 8	+33	+ 3
		Sums	+40	+ 66	+28	− 64
			Alyce + corn	Lesp. + s. pot.	Alyce + corn	
	b	1	− 7	+ 29	− 5	− 70
		2	−16	+ 12	+ 4	− 36
		3	−64	+ 79	−15	−237
		4	+22	+ 13	− 1	− 5
		5	+ 1	+ 37	−13	− 86
		Sums	−64	+170	−30	−434
			Lesp. + s. pot.	Alyce + corn	Lesp. + s. pot.	
B	a	1	+13	− 29	+ 9	+ 80
		2	+20	+ 2	+ 5	+ 21
		3	−25	+ 23	+ 0	− 71
		4	+12	− 9	+22	+ 52
		5	+13	+ 28	− 6	− 49
		Sums	+33	+ 15	+30	+ 33
			Lesp. + corn	Alyce + s. pot.	Lesp. + corn	
	b	1	− 1	+ 14	−18	− 47
		2	+24	+ 9	−10	− 4
		3	+27	+ 35	+19	− 24
		4	+ 4	+ 3	+22	+ 20
		5	+ 9	+ 38	+ 4	− 63
		Sums	+63	+ 99	+17	−118

It appears from these differences that lespedeza excelled alyce hay and that sweet potato meal excelled corn in maintaining liveweight.

In terms of single differences $(P_1 - 2P_2 + P_3)$ the sum of squares for the two sets of factors becomes:

$$\frac{(-413)^2}{20} = 8{,}528 \qquad \frac{(521)^2}{20} = 13{,}572$$

The error sum of squares is $(-23)^2 + (82)^2 \ldots (-63)^2 - 1/5\ (-64)^2 + (-434)^2 + (33)^2 + (-118)^2 = 70{,}850$

Source of variance	*Degrees of freedom*	*Sum of squares*	*Mean square*	*F*
Alyce vs. lesp.	1	8,528	8,528	1.9
S. pot. vs. corn	1	13,572	13,572	3.1
Error	16	70,850	4,428	.....

In this test neither sets of factors are significantly different at the five per cent level. The size of the F value for sweet potato meal vs. corn might lead the experimenter to be suspicious that a real difference did exist and cause him to repeat the experiment in an effort to determine the accuracy of the first information secured.

SUMMARY AND CONCLUSIONS

An efficient method of using the double reversal experimental design to answer two questions with a limited number of cows is presented. The design makes it possible to compare two roughages and also two concentrate mixtures in the same feeding trial. The appropriate use of this design is where previous knowledge or experience indicates that the first degree interaction is unimportant, *i.e.*, that differences between the concentrate mixtures will be approximately the same while on each roughage.

Appropriate tests for measuring the differences based on a 2×2 factorial design are presented. These tests utilize the analysis of variance or the t-test with identical results. Examples given include milk yields showing significant differences between both roughages and concentrates, and liveweight changes, with non-significant differences between each set of factors.

REFERENCES

(1) Cochran, W. G., Autrey, K. M., and Cannon, C. Y. A Double Change-Over Design for Dairy Cattle Feeding Experiments. Jour. Dairy Sci., 24: 937–951. 1941.

(2) Brandt, A. E. Tests of Significance in Reversal or Switchback Trials. Iowa Expt. Sta. Res. Bul. 234. 1938.

(3) Snedecor, G. W. Statistical Methods. pp. 336–361. Collegiate Press, Ames, Iowa. 1938.

(4) Yates, F. The Design and Analysis of Factorial Experiments. Imp. Bur. Soil Sci., Tech. Commun. 35. 1937.

ANNOUNCEMENT

Thirty-Ninth Annual Meeting
of
The American Dairy Science Association
Columbus, Ohio, June 20–22, 1944

NOTE REGARDING THE PROGRAM

In planning the program for the Columbus meeting of the American Dairy Science Association, the officers and members of the Program Committee are endeavoring to focus the program upon the problems of America at War and the problems following, during the peace to come. In order to formulate a program of the most usefulness and interest, it is essential that the committee receive the titles of proposed papers at as early a date as possible, but certainly not later than April 1. Titles will be carefully considered by the committee and abstracts of the papers, which, unless otherwise stated, are assumed to be not longer than 12 minutes, must be in the hands of the Chairman, Professor H. P. Davis, Dairy Husbandry Department, University of Nebraska, Lincoln 1, Nebraska, not later than June 1.

TENTATIVE PROGRAM OUTLINE

Tuesday, June 20, 1944

Morning Session

Welcome by representative of the host institution.
Response and address by President Dahlberg.
Address by guest speaker.

Afternoon Session

Sectional meetings.
4:00 P.M. Section business meetings.

Wednesday, June 21, 1944

Morning Session

Section meetings.
11:00 A.M. Section business meetings.

Afternoon Session

General session on post war problems.

Thursday, June 22, 1944

Morning Session

Joint symposium—Production and Extension Sections.
Manufacturing symposium.

Afternoon Session

General session on Latin American Dairying.
3:30 P.M. Association business meeting.

Evening Session

Annual Banquet.

H. P. DAVIS
Chairman, Program Committee

NEW PROCEDURE TO NOMINATE CANDIDATES FOR OFFICE

In accordance with the provisions of the revised constitution adopted last year, your president has appointed a committee on nominations whose names appeared in the January issue of the Journal and are again listed below. It is the duty of this committee of which J. H. Frandsen is chairman to send its report to the secretary not later than April 1. The secretary will then mail out the ballots to secure a final vote before the annual meeting. The results of the election will be announced at the annual meeting.

As a member of the Association, it is your obligation to express your wishes for officers and directors to any member of this committee. Two candidates for vice-president and four for directors will be nominated. The present vice-president automatically becomes president. The committee on nominations will give consideration to the wishes of the members as expressed by correspondence and will also consider additional candidates. They have been advised by your president to study the list of past officers and directors (see pages 803–804 of the August Journal) to plan to secure good geographic distribution and to recognize the desirability of representation from all lines of activity of our members. This information has been given in detail to the committee for their guidance.

It was the intention of our Association to make the election of officers just as democratic as possible, and this can be done only if members do promptly give their opinions freely to the committee on nominations.

A. C. DAHLBERG, *President*

COMMITTEE ON NOMINATIONS

J. H. FRANDSEN, *Chairman*
H. A. RUEHE
J. F. KENDRICK
C. N. SHEPARDSON
H. P. DAVIS

VOLUME XXVII FEBRUARY, 1944 NUMBER 2

JOURNAL OF DAIRY SCIENCE

Published by the
AMERICAN DAIRY SCIENCE ASSOCIATION
R. B. STOLTZ, Sec.-Treas.
Ohio State University, Columbus, Ohio

ABSTRACTS OF LITERATURE

T. S. SUTTON, Editor
Columbus, Ohio

MILK AND MILK PRODUCTS

Published in cooperation with
INTERNATIONAL ASSOCIATION OF ICE CREAM MANUFACTURERS
R. C. HIBBEN, 1105 Barr Bldg., Washington, D. C., Exec. Sec.

INTERNATIONAL ASSOCIATION OF MILK DEALERS
R. E. LITTLE, 309 W. Jackson Blvd., Chicago, Illinois, Exec. Sec.

Editorial Committee

H. H. SOMMER, Madison, Wisconsin, A. D. S. A.
G. D. TURNBOW, Oakland, California, I. A. I. C. M.
HAROLD PRATT, Philadelphia, Pennsylvania, I. A. I. C. M.
W. D. DOTTERRER, Chicago, Illinois, I. A. M. D.
A. J. POWERS, Brooklyn, New York, I. A. M. D.

CONTENTS

Bacteriology
Breeding
Butter
Cheese
Chemistry
Concentrated and dry milk; by-products
Diseases
Feeds and feeding
Food value of dairy products
Herd management
Ice cream
Milk
Miscellaneous
Physiology

THE SCIENCE PRESS PRINTING COMPANY LANCASTER, PA.

PUBLICATIONS AND ABSTRACTORS

EDITORS

Dahle, C. D., Dahlberg, A. C., Horrall, B. E., Petersen, W. E., Tracy, P. H. and Weckel, K. G.

ABSTRACTORS

Archibald, J. G.
Babcock, C. J.
Bennett, F. W.
Berggren, Ruth E.
Burgwald, L. H.
Bushnell, L. D.

Call, A. O.
Cole, W. C.
Cone, J. F.
Coulter, S. T.

Doan, F. J.
Dorsey, L. M.
Downs, P. A.

Ely, Fordyce
Erb, J. H.
Espe, D. L.

Frazier, W. C.
Fuller, S. A.

Glick, D. P.
Goss, E. F.

Henderson, J. L.
Huffman, C. F.

Irvine, O. R.

Josephson, D. V.

Lucas, P. S.
Lush, J. L.

Marquardt, J. C.
Mueller, W. S.

Price, W. V.
Pyenson, Harry

Reece, Ralph P.
Roberts, Henry E.

Thomsen, L. C.
Trout, G. M.

Waugh, R. K.
Webb, B. H.
Weckel, K. G.
Weiser, H. H.
White, G. C.

Yale, M. W.

JOURNALS

American Butter Review
American Milk Review
American Journal of Diseases of Children
American Journal of Physiology
American Journal of Public Health
Archives of Pediatrics
Australian Journal of the Council for Scientific and Industrial Research

Biochemical Journal

Canadian Dairy and Ice Cream Journal
Canadian Journal of Public Health
Canadian Journal of Research
Certified Milk
Cornell Veterinarian

Dairy Industries
Dairy World

Endocrinology

Food in Canada
Food Industries
Food Manufacture
Food Research

Ice and Refrigeration
Ice Cream Field
Ice Cream Review
Ice Cream Trade Journal
Industrial and Engineering Chemistry

Journal of Agricultural Research
Journal of Agricultural Science
Journal of American Medical Association
Journal of American Veterinary Medical Association
Journal of Animal Science
Journal of Bacteriology
Journal of Biological Chemistry
Journal of Dairy Research
Journal of Dairy Science
Journal of Endocrinology
Journal of Experimental Medicine
Journal of General Physiology
Journal of Genetics
Journal of Heredity
Journal of Industrial and Engineering Chemistry
Journal of Infectious Diseases
Journal of Milk Technology
Journal of Nutrition
Journal of Pathology and Bacteriology
Journal of Physical Chemistry
Journal of Physiology
Journal of Veterinary Research

Lancet
Le Lait

Milk Dealer
Milk Industry
Milk Plant Monthly

National Butter and Cheese Journal
New Zealand Journal of Science and Technology

Oil and Soap

Pacific Dairy Review
Proceedings of Society of Experimental Biology and Medicine

Refrigerating Engineering

Scientific Agriculture
Southern Dairy Products Journal

SPECIAL PUBLICATIONS

Federal Dairying and Bacteriological Establishment, Liebefeld, Berne, Switzerland

International Association of Ice Cream Manufacturers
International Association of Milk Dealers

National Institute for Research in Dairying, Reading, England
New York Association of Dairy and Milk Inspectors

Prussian Dairy Research Institute, Kiel, Germany

State Agricultural Colleges and Experiment Stations

The Royal Technical College, Copenhagen, Denmark

United States Department of Agriculture

ABSTRACTS OF LITERATURE

BOOK REVIEW

27. Drying and Dehydration of Foods. HARRY W. VON LOESECKE, Bureau of Agricultural Chemistry and Engineering, U. S. Department of Agriculture. Published by Reinhold Publishing Corporation, 330 West Forty-second Street, New York. Consists of 10 chapters, glossary of terms, patent list, index. 302 pages. $4.25.

The tremendous wartime use of dehydration as a means of enabling efficient preservation and movement of foods in the light of packaging and transportation problems has led to many developments in the art. The dairy industry has experienced a phenomenal increase in dehydration in its own field. A knowledge of the processes and developments in other food product fields is important to workers in the dairy industry because of a potential utilization of milk in dehydrated processed foods. The book, Dehydration of Foods, is written in an excellent review manner, devoid of quotations and citations. The references necessary to the subject are listed separately at the end of each chapter. Types of dehydrators includes illustrations of the principal types of units employed in the dehydration industry. A short resume is included in this chapter on the air, heat, moisture relationships of a tunnel dryer. Other chapters are: Sun drying and dehydration of fruits; Dehydration of Vegetables; Dehydration of Eggs; Milk and Butter; Dehydration of Meat, Fish, and Beef Blood; Plant Sanitation; Costs of Dehydration; Nutritive Value of Dried and Dehydrated Foods; Packaging and Storage; Methods of Analysis, and Reconstitution of Dehydrated Foods. The section on dehydration of milk contains no information not available in recognized dairy texts. No discussion is included of specific techniques and problems of roller drying of skim and whole milk, nor of the quality standards for dried milk. The material in this chapter is brief in the light of the importance of the industry. The discussion in the text is well organized, and easily readable, and enables a ready grasp of principal methods in food dehydration. It will be an excellent book for those in the dairy industry seeking a good review of other branches of the food dehydration industry, and their methods. K.G.W.

BREEDING

28. An Analysis of Milking Shorthorn Records. W. L. GAINES. Ill. Agr. Expt. Sta. Bul. 498. Aug., 1943.

Because milk records for Milking Shorthorn cows had never been analyzed in the same way as those for other breeds, data from Volumes 9 to 23 (1924–1938) of the Milking Shorthorn Year Book were collected and studied. The records were studied as a whole and in groups according to

number of milkings daily, length of calving interval, and length of record. Only records which included age of cow at calving, length of record, milk-fat yield, and fat percentage in addition to milk yield were studied. Milk-energy yield was computed by the usual formula. For the 6,311 records the average yield was 8,337 pounds of milk, 330 pounds of fat, and 8,285 pounds of FCM, and the average fat percentage was 3.97. The subgroups differed greatly in average milk yield, milk-fat yield, and FCM yield, but differed very little in average fat percentage. For the records as a whole, the correlation between fat percentage and milk yield was -0.217; between fat percentage and fat yield, $+0.106$; and between fat percentage and FCM yield, -0.026 (not significant). Similar correlations were found in each of the subgroups. When the change in yield between 3.0% fat and 5.5% fat was expressed by a straight line, milk yield showed a decrease of about 30%, fat yield increase about 30%, and FCM showed very little change. These records afforded an opportunity to check the age-correction factors previously used for Milking Shorthorns, which were based on records of the breed up to June 1, 1920. The records reported here show a distinct shift toward earlier maturity, amounting to 6 months; and the age-correction factors need to be adjusted accordingly. There is no way of knowing whether this earlier maturity represents a change in the dairy qualities of the breed or a change in management of the cows. Actually age correction is probably simply an indirect allowance for live weight increases with age. A system of milk-yield correction based on live weight would be biologically more sound than an age-correction system, at least for cows less than 13 years old. The season in which a cow calved had an appreciable effect on FCM yield. In general August calvers had the lowest yield and November calvers had the highest. Certain of the records were studied to discover the difference between FCM yields of cows milked three times a day and of cows milked twice a day. The records of a typical group showed that cows milked three times a day exceeded in yield those milked twice a day by 39%. The standard for dairy cows of the Bureau of Dairy Industry is that cows milked three times a day should outyield those milked twice by 24%, so it appears that Milking Shorthorns respond well to the more favorable conditions associated with three milkings daily. A new table of age-correction factors for the Milking Shorthorns is given together with notes explaining its adaptation for use with other dairy breeds.

11 figures, 8 tables. J.G.A.

BUTTER

29. The 50–45–40 Method of Making Butter. G. H. Wilster, Oreg. State Col., Corvallis. Natl. Butter and Cheese Jour., *34*, No. 11: 16. Nov., 1943.

See Abstract No. 534, page A229, Vol. *26*, No. 12, Dec., 1943.

W.V.P.

CHEESE

30. What About the Tentative New U. S. Cheese Grades? WALTER V. PRICE, Univ. of Wis., Madison. Natl. Butter and Cheese Jour., *34*, No. 11: 8. Nov., 1943.

The tentative U. S. grades on cheddar cheese are unnecessarily complicated in defining grades for the primary market and unjustified in extending similar definitions and grade names to the consumer trade in cured cheese. These tentative grades should be carefully studied by graders, trade associations, consumer groups and commercial organizations and public hearings should be held by the Food Distribution Administration to review and, if necessary, revise these grades. W.V.P.

31. Milk Filtering in the Cheese Factor. RAYMOND MIERSCH AND WALTER V. PRICE, Univ. of Wis., Madison. Natl. Butter and Cheese Jour., *34*, No. 12: 26. Dec., 1943.

Filtration of milk at the cheese factory does not justify the acceptance of insanitary milk. The capacity of a given filter varies with the condition of the milk, such as its cleanliness, viscosity, acidity, fat and curd content. Preheating of the milk is sometimes used to increase filter capacity. Efficiency of a given filter is affected by the type of pad used, the re-use of washed pads and the volume and the sanitary condition of the milk. Clarification by centrifugal force is being used effectively in the Swiss cheese industry. Costs of filters vary from $150 to $600 for commonly used types and up to $1300 for larger models; filter pads cost from 35 cents to about $1.50 per day. Manufacturers who try to wash and re-use a filter pad take an unjustified risk. W.V.P.

CHEMISTRY

32. Pectin as an Emulsifying Agent. Comparative Efficiencies of Pectin, Tragacanth, Karaya, and Acacia. HARRY LOTZKAR AND W. DAYTON MACLAY. Western Regional Res. Lab., U. S. Dept. Agr., Albany, Calif. Jour. Indus. and Engin. Chem., Indus. Ed., *35*, No. 12: 1294. Dec., 1943.

The emulsifying ability of pectin is compared to that of gums tragacanth, karaya and acacia. The importation of these gums has been curtailed by shipping difficulties. The study was made of aqueous emulsions of olive, cottonseed and mineral oils under various conditions of acidity, ratio of oil to water and concentration of agent. Changes in the emulsions were followed by measuring the pH, viscosity, and specific interfacial area of the dispersed oil. In general the tragacanth stabilized emulsions were coarse and viscous, the acacia emulsions were fine and fluid, the karaya emulsions were gelatinous and the pectin emulsions were fine and viscous. The varia-

tion of emulsifying efficiency with acidity and from oil to oil limited generalizations that could be made on the comparative emulsifying efficiencies of pectin and the gums. B.H.W.

33. Refractive Index Nomograph for Liquid Fatty Acids. D. S. Davis, Wyandotte Chemicals Corp., Wyandotte, Mich. Jour. Indus. and Engin. Chem., Indus. Ed., *35*, No. 12: 1302. Dec., 1943.

Data in the literature covering the variation in refractive index with temperature, in the liquid state, for the normal saturated fatty acids from caproic to stearic, enabled the author to construct the accompanying chart for determining the refractive indices of these acids at any temperature in the range of applicability. B.H.W.

CONCENTRATED AND DRY MILK; BY-PRODUCTS

34. Dry Whole Milk—The Relation of Lecithin to Its Keeping Qualities. C. D. Dahle and D. V. Josephson, Dairy Dept., Penn. State Col., State College, Pa. Ice Cream Trade Jour., *39*, No. 11: 22. Nov., 1943.

The stability of dry whole milk toward the development of oxidized flavor during storage was found to be improved by the partial removal of lecithin normally found in the fat globule adsorption layer. This removal was accomplished by separating the milk, churning the cream and rendering the butter. The resulting butter oil was remixed with the skimmilk before homogenizing, concentrating and drying on a small open roll drier. In one trial the fluid skimmilk was supercentrifuged before mixing with the butter oil. This apparently removed some of the lecithin found in the skimmilk and the dry product exhibited further improvement in keeping quality. Lecithin contents of the dry milks were 0.753, 0.480, and 0.366% (on the basis of the fatty extracts) respectively, for the control product, the butter oil and normal skimmilk product and the butter oil and supercentrifuged skimmilk product.

Improvement in keeping quality of dry whole milk resulting from a partial removal of lecithin was noted in both normal and nitrogen packed containers. F.J.D.

DISEASE

35. Septic Sore Throat Epidemic at School. Fred W. Caudill, M.D., M.P.H. Dir., Div. of Communicable Diseases, and Melville A. Meyer, State Milk Sanit., Bur. of Foods, Drugs and Hotels, State Dept. of Health of Kentucky. Jour. Milk Technol., *6*, No. 4: 221. July–Aug., 1943.

A study is reported of two outbreaks of septic sore throat, which occurred among the students, teachers and other employees of the school.

Hemolytic streptococci were cultured from specimens of milk taken from the udders of 3 of the 10 cows in the herd furnishing milk for the school. All of the 10 cows showed the presence of *Staphylococcus aureus* or *albus* in the specimens of milk. The *Staph. aureus* were hemolytic in every instance.

It was thought that one of the boys, who helped with the milking and who reported to the infirmary with a sore throat, which appeared to be an extremely septic throat, was the source of the infection. A period of 7 days had elapsed between his reporting to the infirmary and the occurrence of the first large number of onsets. Improper cleansing and sterilization of milk utensils, milker's hands, cows' udders, and inadequate cooling were thought to be factors contributing to the outbreak. L.H.B.

36. Newer Methods for Control of Mastitis. G. R. SPENCER, Univ. of Wis., Madison. Natl. Butter and Cheese Jour., *34*, No. 11: 48. Nov., 1943.

Find infected cows by regular use of strip cups and the Hotis test; segregate them, in separate barns if possible; and use separate teat cups on infected cows. Keep barns clean. Feed less grain to reduce production of infected cows. Milk cows regularly and thoroughly. Medicinal treatments for mastitis as yet are not wholly effective. W.V.P.

37. Some Results with Mastitis at Michigan State College. R. C. HORWOOD, C. F. CLARK, AND C. S. BRYAN. Reprint from Mich. Agr. Expt. Sta. Quar. Bul., *26*, No. 1: 43–50. Aug., 1943.

At the start of this program in 1932 there were 56 cows in milk in the college herd. Of these 53.5% had infectious mastitis. In November, 1941, the herd passed its first completely negative test. During the following 13 months to January 1, 1943, there have been nine months without any reacting cows. Acute systemic infection has increased and resulted in an increased loss from death or functional use of the udder. The results in lowering infection in the herd were obtained by culling the infected cows, preventing the spread of the organism, and by recovery by treatment of 16 mastitis-infected animals by lactovaccine and tyrothricin. Thirty-six non-infected cows produced 45% of the milk and 44% of the butterfat from the fore quarters. A reduction of 32% in milk and 32% in butterfat was found in a similar study of 9 cows with opposite-infected and non-infected rear quarters. A comparison of microscopic method, chloride determination, physical examination of udder, leucocyte determination, thybromol test, physical examination of milk and culture of milk on blood agar plates, of testing for infectious mastitis on individual quarters, from November, 1932, to February, 1937, on the college herd led to the adoption of the monthly microscopic system of testing. There was no significant variation found in the milk nor udders of three mastitis-infected cows receiving 50

one-hour daily treatments with the short-wave diathermy as compared with three untreated mastitis-infected cows. Lactovaccine was found to correct 26% of the cows positive to streptococci infection. Fifty per cent of the animals responding did so from four treatments, others required up to 12. Treatments beyond that number gave no correction. The study did not indicate that an immunity to infection was developed in non-infected cows. Nine cows treated with tyrothricin became negative to infectious mastitis. A temporary effect which resulted in lower milk production and the production of abnormal milk was observed. Four animals that were treated within three weeks following infection all maintained or showed no increase in production in the lactation following treatment. Only one animal with long-standing infection responded in this manner. Three of the remaining four animals with long-standing infection (10 months to nine years) later developed acute systemic mastitis and died or lost the functional use of the udders. Three other cows in the herd not previously infected were lost during the same period. J.G.A.

FEEDS AND FEEDING

38. Relationship between Fat Content of Dairy Grain Mixtures and Milk and Butterfat Production. C. F. Monroe and W. E. Krauss. Ohio Agr. Expt. Sta. Bul. 644. 40 pages, illus. Aug., 1943.

Five feeding trials involving a total of 128 cows were conducted in order to determine the effect of different levels of fat in the grain mixtures on milk and butterfat production. The grain mixtures used contained only natural and by-product feeds commonly used in practice. The various fat levels were obtained, with some slight exceptions, by supplementing basal mixtures with either 41% (expeller) soybean oil meal, both with and without ground soybeans, or 44% (browned extracted) soybean oil meal. The continuous type of feeding trial was employed in all five of the trials summarized. With the exception of six Jerseys used in one trial in the second series, the cows were all Holsteins of fairly high milk-producing ability. In the first series of two trials involving 70 cows fed on three levels of fat, the average production of 4% (F.C.M.) milk per 30 days was: pounds, high-fat, 932.1; medium-fat, 921.1; and low-fat, 923.9. In the second series of three trials involving 58 cows fed the two levels of fat, the average production of 4% (F.C.M.) milk per 30 days was: pounds, high-fat, 1,077.5; low-fat, 1,054.3. The butterfat tests and liveweight gains were apparently not affected by the fat levels in the feed. There was also no noticeable difference in palatability in the grain mixtures used. There was no noticeable difference in their effect on the general health and condition of the cows or in preventing or causing udder trouble. Under the conditions of these trials, no significant differences were observed in the production of milk,

butterfat, or 4% (F.C.M.) milk or in the general health of milking cows from the feeding of practical grain mixtures ranging in average fat percentage from 4.89 to 2.69.

19 tables, 5 figures, 23 references. J.G.A.

39. The Use of Dried Whey and Blood Meal in the Raising of Calves on Limited Amounts of Milk. I. L. HATHAWAY, G. W. TRIMBERGER, AND H. P. DAVIS. Neb. Agr. Expt. Sta. Res. Bul. 132. 19 pages. Oct., 1943.

Fifty grade Holstein heifer calves were successfully raised from approximately three weeks to six months of age on alfalfa hay, a grain mixture, a vitamin concentrate, and various amounts of skim milk, supplemented with a mixture composed of 3.2 parts of dried whey to one part of blood meal. Six and eight-tenths pounds of this mixture were used to replace 50 pounds of skim milk. The milk was fed at 50-, 100-, 150-, 200-, 250-, and 300-pound levels. The calves in five of the lots made an average daily gain of approximately 1½ pounds per head for 21 weeks. There was no statistically significant difference among the average gains in weight made by five of the six lots of calves. Three hundred pounds of skim milk alone produced cheaper gains than 50 pounds of skim milk supplemented with 34 pounds of the whey-blood-meal mixture. It was concluded that 6.8 pounds of a mixture composed of 3.2 parts of dried whey and one part of blood meal is a satisfactory substitute for 50 pounds of skim milk in the feeding of healthy dairy calves, which are approximately three weeks of age and which weigh not less than 104 pounds. Thirty-four pounds of this whey-blood-meal mixture can be fed, without serious effects, in 30 days even though as much as 1.8 pounds are fed daily for a few days. Healthy, vigorous dairy calves can be satisfactorily raised from three weeks to six months of age on as little as 50 pounds of the skim milk if it is properly supplemented with dried whey, blood meal, alfalfa hay, a grain mixture, and a vitamin concentrate. Labor can be saved in the raising of dairy calves by replacing the pail feeding of milk over a long period with a few weeks of milk feeding followed by a suitable grain mixture, alfalfa hay, and a vitamin concentrate. With the prices of feeds as quoted herein, the feed cost of raising calves on milk only may be less than when the milk is replaced by this whey-blood-meal mixture. However, when the labor cost is considered in connection with the feed cost, the additional expense of using the substitute mixture will not be prohibitive. Dried whey and blood meal can be utilized as a means of diverting milk from calf feeding to human food and to other uses.

J.G.A.

40. The Nutritive Value of Korean Lespedeza Proteins and the Determination of Biological Values of Proteins for Growing Dairy

Heifers. E. W. SWANSON AND H. A. HERMAN. Mo. Agr. Expt. Sta. Res. Bul. 372. 68 pages, illus. Aug., 1943.

An investigation was made of the utilization of the crude protein ($N \times 6.25$) of Korean lespedeza hay and seed and various other feeds by growing dairy heifers. Biological values of the various proteins were determined, and net protein values and other measures of the nutritive value of the proteins were calculated. The net utilization of proteins from lespedeza hay, alfalfa hay, dried skimmilk, corn, lespedeza seed, soybean oil meal, and combinations of lespedeza hay with corn, milk, or soybean oil meal was not significantly different for dairy heifers when they were fed at a 10% level. The feeds were ranked according to the biological value of their proteins as follows: lespedeza hay, corn and lespedeza hay, milk and lespedeza hay, alfalfa hay, corn, soybean oil meal, soybean oil meal and lespedeza hay, lespedeza seed, and dried skimmilk. It was concluded that the quality of the absorbed proteins from Korean lespedeza hay or seed was equal to the quality of the absorbed proteins from milk, corn, alfalfa hay, or soybean oil meal for growing dairy heifers. The digestibility of the crude protein of lespedeza hay was shown to be relatively low, and the high lignin content of lespedeza leaves was revealed as a possible explanation of the poor protein digestibility. Highly lignified late-cut lespedeza hay was shown to be of very low nutritive value, the digestibility of all of its nutrients being greatly depressed. Digestion coefficients were determined for all of the nutrients of intermediate-cut lespedeza hay, late-cut lespedeza hay, and ground lespedeza seed.

12 figures, 16 tables, 110 references. J.G.A.

HERD MANAGEMENT

41. Faster Milking. W. E. PETERSEN. Univ. of Minn. Agr. Ext. Serv. Folder 119. Illus. June, 1943.

The technique of faster machine milking is briefly set forth with reasons for the several steps. Rules are as follows: (1) stimulate the cows to let down their milk one minute before putting on the machine; (2) operate the milking machine according to the manufacturer's directions; (3) strip the cows by machine; and (4) do not leave the machine on the cow after milk stops flowing. J.G.A.

ICE CREAM

42. The Preparation and Use of Invert Syrup in the Manufacture of Ice Cream. E. L. FOUTS, L. E. MULL AND T. R. FREEMAN, Univ. of Fla., Gainesville. Fla. Agr. Expt. Sta. Bul. 393. Sept., 1943.

Invert syrup of 70% solids was made using various acids and heating times. It was found that a satisfactory syrup could be made by boiling for

30 minutes 100 grams of tartaric acid, 100 pounds of sugar and 44 pounds of water. Citric acid in the same amount was found to be equally satisfactory. Three pints of lemon juice in the same formula produced equally satisfactory results and can be substituted for the tartaric and citric acids which are difficult to obtain. The invert syrup will keep well at room temperature.

In ice cream the invert syrup was found to be equal in sweetening value to the same amount of cane sugar and it is recommended that 25 to 50% substitutes of cane sugar can be made in ice cream, but where the latter amount is used the serum solids of the mix should be increased 1%.

C.D.D.

MILK

43. Is It Desirable to Simplify and Unify Our Milk Quality Program. From the Viewpoint of the Health Officer. N. O. GUNDERSON, Commr. of Health, Rockford, Ill. Jour. Milk Technol., *6*, No. 4: 225. July–Aug., 1943.

A four-point test system in use in Rockford, Illinois, is described. This system, after an initial inspection of the farm, shifts the point of observation with reference to quality control from the farm to the receiving dock or city plant. The four-point test used for judging the quality of milk is (1) Sediment test, (2) Direct microscopic (or Reduction test), (3) Phosphatase test, and (4) Swab test, together with other simple observations made at the receiving or processing plant. Under this system farm inspections are limited to the small number of non-compliance milk producers.

The cost of supervising this plan is approximately 0.155 cents per gallon of milk.

Preliminary results of this system of inspection at Rockford for the first five months of 1943 are given.

Some advantages given for this system are as follows:

1. A method of unifying milk control procedures.
2. Quickly locates apparent source of poor milk.
3. A means of culling out low-producing mastitis cows.
4. Minimizes time-consuming routine farm inspection.
5. Emphasizes safety, quality, and flavor of milk—not esthetic scoring of milk control.
6. Is apparently applicable to not only fluid milk, but to cream, butter, cheese, evaporated and dried milk quality control procedures.
7. Conserves manpower so essential in the war effort.

A suggestion is made that a National Allied Dairy Products Council be formed for the purpose of formulating specific recommendations that will simplify and unify the many varied milk control procedures applied to fluid milk, cheese, cream, butter, ice cream, evaporated and dried milk. L.H.B.

44. Homogenized Milk and Public Health. G. M. TROUT, Mich. Agr. Expt. Sta., East Lansing, Mich. Jour. Milk Technol., *6*, No. 4: 214. July–Aug., 1943.

The history and development of homogenized milk are given together with a very good bibliography on the subject.

Some of the facts regarding homogenized milk and particularly those having a public health significance are discussed. The factors considered are:

1. Homogenized milk must be a pasteurized product. Raw homogenized milk will develop a rancid flavor in a very short time.

2. Homogenized milk may be pasteurized at a higher temperature and maintained for a longer holding period than regular pasteurized milk. There is no cream line on homogenized milk, which will be destroyed by high pasteurization temperatures.

Homogenized milk cannot be mixed with raw milk. Rancid flavor will develop. Only clean milk may be used for homogenizing, otherwise, sedimentation is a possibility. Homogenization, when applied to milk, has a tendency to make the processor more "milk conscious" as it requires more care in processing and merchandising than does unhomogenized milk. Homogenized milk is a factor in developing increased milk consumption, due principally to the fact that homogenization protects against oxidized flavor.

L.H.B.

45. Milk House Construction, Equipment and Maintenance. H. A. BENDIXEN, State College of Wash., Pullman, Wash. Jour. Milk Technol., *6*, No. 3: 175. May–June, 1943.

Studies were made at State College on milk house construction and equipment suitable for Washington conditions.

Location, size and construction of milk houses are discussed together with types of materials best suited.

L.H.B.

46. Market Homogenized Milk in Philadelphia. BERNARD SPUR, Milk Res. Labs., Children's Hosp. of Philadelphia, Philadelphia, Pa. Jour. Milk Technol., *6*, No. 5: 266. Sept.–Oct., 1943.

That the sanitary quality of the homogenized milk produced in Philadelphia is exceptionally good is shown by the study made at the Milk Research Laboratories, Children's Hospital of Philadelphia, during the eight months period from December, 1940, to September, 1941. Samples were taken each month during this period, with the exception of January and March. The samples were purchased at the distributing platforms of the milk plants.

Of 34 plants producing Grade A homogenized milk, 26 of them had average counts under 8,000. This represented 96% of the total Grade A

homogenized milk consumed in Philadelphia. Only three plants had average counts over 25,000.

There were 36 plants producing Grade B homogenized milk, and of these plants, 22 had average bacterial counts under 8,000. This represented 89% of the Grade B homogenized milk consumed. All but two of the plants had average counts under 50,000 and only one was over 100,000. Twelve of the dairies were known to pasteurize before homogenizing, while three were known to homogenize before pasteurizing.

The bacterial counts obtained in these plants seemed to indicate that the question of getting low counts is more dependent on the sanitary conditions in the plant (and probably on the type of bacteria in the raw milk supply) than on the order of the processes. Of the twelve plants pasteurizing before homogenizing, only one had an average bacterial count over 5,000, and that was an average count of 18,200 on the Grade B milk. The average bacterial count on this plant's Grade A milk was 4,018. Of the three plants pasteurizing after homogenization, two of them had very low counts on both Grade A and B milks (less than 1,400). The other plant had average counts of 15,338 on the Grade A and 11,681 on the Grade B.

The average curd tension of the Grade A milks was 11.6 gms. and on the Grade B was 11.2 gms. The curd tension for the Grade A milks ranged from 5.3 gms. to 18.4 gms., while for the Grade B milks it ranged from 4.6 to 15.9 gms. L.H.B.

47. A Ropy Milk Outbreak Caused by a Thermoduric Micrococcus. C. C. PROUTY, Div. of Dairy Husbandry, Wash. Agr. Expt. Sta., Pullman, Wash. Jour. Milk Technol., *6*, No. 5: 263. Sept.–Oct., 1943.

An outbreak of ropy milk occurring in commercially pasteurized milk was studied. An organism closely related to *Micrococcus freudeneichii* was isolated and its source was traced to one of the dairies furnishing milk to the pasteurizing plant. When milk from this producer was excluded, no further ropiness was experienced in the pasteurized product.

Some cultures of the organism were found to survive a pasteurization temperature of 143° F. for 35 minutes, while in other instances 20 minutes at that temperature was sufficient to render the organism nonviable.

The organism had the ability to cause rapid development of ropiness with an abnormal flavor and odor, followed by active proteolysis. L.H.B.

48. The Connecticut Three-Point Laboratory Program as an Aid to Control of Pasteurized Milk. FRIEND LEE MICKLE AND EARLE K. BORMAN, Dir. and Asst. Dir., Bur. of Labs., Conn. State Dept. of Health, Hartford, Conn. Jour. Milk Technol., *6*, No. 4: 231. July, Aug., 1943.

A three-point program for determining the quality of pasteurized milk

was started in 1937 after a state-wide survey. The tentative standards set were:

"1. Direct microscopic clump count limits:
Grade A pasteurized milk—200,000 per ml.
Grade B pasteurized milk—500,000 per ml.
2. Coliform organisms: Absent in 0.1 ml.
3. Phosphatase test: Less than 0.05 mg. phenol."

The coliform standard was changed to *absent in 1.0 ml.* in January, 1941.

The use of these standards has not worked a hardship on dealers in good quality milk.

The direct microscopic clump count on pasteurized milk has proven satisfactory in easily classifying milk as either good or bad and with greater rapidity than the agar plate count. In 1939 only 4.1% of the Grade A samples had counts in the borderline range between 200,000 and 300,000, while only 6.5% of the Grade B samples had counts in the borderline range between 500,000 and 1,000,000.

During the five-year period 77.4% of the samples (more than 15,000) met the tentative standards of 200,000 for Grade A and 500,000 for Grade B pasteurized milk by the direct microscopic clump count; 75.6% met the tentative standard for coliform organisms; and 92.7% met the tentative standard for the phosphatase test.

The advantages of the three-point program for determining the quality of pasteurized milk were given as follows:

"1. The direct microscopic clump count presents a more nearly true picture of the bacteriological quality of a milk supply than does the plate count, and provides a much more rapid, more complete, and hence more effective laboratory service to the control official.

2. Tests for coliform organisms, furnishing evidence of improper handling after pasteurization, yield supplementary information correlating well with the direct count.

3. The phosphatase test yields the only positive information obtainable on the effectiveness of pasteurization, and hence is an indispensable index of probable safety of a supply." L.H.B.

49. Efficiency of Milk Marketing in Connecticut. 5. Economics and Biology of Alternate-Day Milk Delivery. R. G. BRESSLER, JR., E. O. ANDERSON, D. A. CLARKE, JR., AND E. N. BILENKER. Conn. Agr. Expt. Sta. Bul. 247. 60 pages. May, 1943.

Alternate-day delivery of milk has proved to be an effective method of conserving resources. Retail milk truck mileage has been reduced an average of 44% in Connecticut markets. The mileage savings and increased deliveries per stop reduced route time by nearly one-third, which in turn made possibly increased loads and some consolidation of routes. The pro-

gram reduced requirements for gasoline and tires by approximately 40%. Man-hours were reduced 30%, but some of this reduction represented shorter hours per route so that the number of men freed for other employment was only 14% in 1942. Under present conditions annual savings in Connecticut total one million gallons of gasoline, forty-six thousand quarts of oil, and two thousand truck tires, and the elimination of nearly 200 routes.

Monetary savings have not been large in spite of these important reductions in men and materials. In 1941, retail delivery costs in city markets average 4.06 cents per quart. In the spring of 1942 alternate-day delivery costs were approximately 3.70 cents per quart, while a year later, they were 3.61 cents. These savings were offset at least in part by wartime increases in the other costs of milk distribution. If alternate-day deliveries are continued in the post-war period, and if weekly earnings of routemen return to the normal pre-war levels, total delivery costs would be approximately 2.74 cents, a savings of 1.32 cents per quart.

Successful application of alternate-day delivery is dependent on the keeping qualities of milk. Experiments indicate that satisfactory results are obtained where home refrigeration is adequate. P.H.T.

50. Efficiency of Milk Marketing in Connecticut. 6. Truck Costs and Labor Requirements on Milk Delivery Routes. D. A. CLARKE, JR., AND R. G. BRESSLER, JR. Conn. Agr. Expt. Sta. Bul. 248. 39 pages. June, 1943.

In general, costs fell into three categories: overhead costs, fixed operating costs per day, and variable operating costs per mile. Combining overhead and fixed operating costs, the daily costs of operating retail trucks average $1.68 plus $0.44 per mile. For wholesale trucks daily costs averaged $2.46 plus $0.045 per mile. These costs are based on daily delivery conditions and on cost rates representative of the period 1940–41. The costs of operating alternate-day delivery trucks averaged about $7.51 per day plus $0.045 per mile during the spring of 1943.

In greatly simplified form the time required to operate daily retail routes may be represented by 1.2 minutes per quart plus four minutes per mile, while for alternate-day delivery the time requirements are 0.8 minutes per quart plus four minutes per mile. On wholesale routes the time per quart is only 0.35 minutes. Under average conditions of route organization it is possible to handle about 35 quarts per hour on daily retail delivery routes, 60 quarts per hour on alternate-day retail routes and 125 quarts per hour on wholesale routes.

With daily delivery, the average retail driver earned about $44 per week and labor costs averaged about $0.026 per quart. With alternate-day delivery average weekly earnings were $56 (commission basis) while labor costs averaged $0.025 per quart. On salary and commission payments the

weekly earnings averaged $46 and costs $0.025 per quart. On wholesale routes the average cost per quart was between $0.006 and $0.007.

In 1941 truck costs averaged $0.011 per quart, route labor costs $0.026, miscellaneous costs $0.004, and total delivery costs of $0.041 per quart. In 1943 with alternate-day delivery, truck costs averaged $0.007, route labor $0.025, miscellaneous route costs $0.004 and total retail delivery costs $0.036 per quart. In the spring of 1943 wholesale delivery route truck costs average about $0.003 per quart, labor costs $0.006, miscellaneous costs $0.004, and total delivery costs $0.014 per quart. P.H.T.

51. Efficiency of Milk Marketing in Connecticut. 7. Milk Delivery in Rural Connecticut. ALAN MACLEOD AND C. J. MILLER. Conn. Agr. Expt. Sta. Bul. 249. 37 pages. July, 1943.

Opportunities exist for conserving scarce resources in the distribution of fluid milk in rural areas. A study of 12 areas in Connecticut has indicated the size of the savings that might be realized from complete adoption of alternate-day delivery and from the allocation of exclusive territories with deliveries made daily or on alternate days.

In the summer of 1942 about 36 per cent of the daily delivery mileage was being saved. This compares with a maximum estimated potential savings of 45 per cent if all producers were placed on an alternate-day basis.

Adoption of a system of exclusive terrritories would yield potential savings of 38 per cent with daily delivery or of 64 per cent with alternate-day delivery. The present system of alternate-day and daily delivery is saving almost 1.3 million miles yearly in rural Connecticut towns. These savings could be increased to 2.2 million miles by the adoption of exclusive territories in combination with alternate-day delivery. This would produce a savings of about 220,000 gallons of gasoline, 11,000 quarts of oil, and 500 delivery truck tires. The savings in manpower would probably be most important of all as the time released from delivery can be put to more productive uses. P.H.T.

MISCELLANEOUS

52. Post-War Problems of the Locker Industry. SLEETER BULL, Univ. of Ill., Urbana. Natl. Butter and Cheese Jour., *34*, No. 11: 22. Nov., 1943.

The rapid growth of the locker industry has resulted in some troubles caused by poor plant design, improper insulation, under equipment, lack of information in management and operation, and consumer ignorance. The future promises more problems such as the development of household units for home freezing and storage and the possibility of freezing foods in transit at high altitudes. W.V.P.

53. Pyrex Glass Tubing as a Substitute for Metal Milk Pipe in Dairy Plants. G. J. HUCKER AND ROBERT E. THOMAS, N. Y. State Agr. Expt. Sta., Geneva, N. Y. Jour. Milk Technol., *6*, No. 4: 197. July–Aug., 1943.

A study was made under commercial conditions in a plant handling approximately 45,000 pounds of milk daily.

The study revealed that pyrex glass tubing, beaded or flanged, can serve as a substitute for metal milk pipe in dairy plants.

It proved impractical under general operating conditions to dismantle beaded glass tubing daily for cleansing, the same as metal pipe. Some breakage and chipping was experienced when this was done.

It was found that pyrex tubing could be satisfactorily cleaned in an assembled position.

A bacteriological study of glass tubing cleaned and sterilized without disassembling gave results, from a sanitary standpoint, which were satisfactory and comparable to those obtained when metal pipes are dismantled daily and cleaned in the usual manner practical in the dairy industry at present.

The method used for daily cleaning of the glass tubing in the assembled position was as follows:

Rinse milk lines by circulating (1) cold water, (2) a 0.6% solution of an alkali cleanser containing 4.0% of a wetting agent at a temperature not less than 110° F. for at least 20 minutes, (3) clean water rinse at 110° F., (4) hot water rinse at about 190° F. for not less than 15 minutes. Just before use circulate a chlorine rinse solution of at least 100 ppm. strength through the entire milk processing system. Glass tubing and tube joints opened and examined at intervals of two to six weeks using this treatment were found to be in an excellent sanitary condition.

The optimum time interval for disassembling and examining tubing and joints was not determined. It was suggested, however, that the interval should not be greater than two weeks, until further data were secured.

L.H.B.

54. Comparative Educational Background of Dairy Graduates, Sanitary Engineers and Veterinarians in Milk Control. SIDNEY SHEPARD, Birmingham, Ala. Jour. Milk Technol., *6*, No. 4: 235. July–Aug., 1943.

"Since World War I the field of dairy science as it relates to public health has slowly but surely drifted from the dairy college graduates into the hands of sanitary engineers (a division of civil engineering) and veterinarians. Today, only sanitary engineers may hold commissions as milk sanitarians with the United States Public Health Service, and only veterinarians are deemed qualified to exercise sanitary supervision over the

production and manufacture of all products of 'bovine origin' being used by our armed forces."

From a study made of the curricula of five dairy colleges, four veterinary colleges and five schools of sanitary engineering ("In each instance the colleges studied were those generally accepted as outstanding in their particular field."), it was obvious that the dairy colleges were doing a good job in educating men for a career in milk sanitation. In every instance they had courses in dairy manufacture, animal husbandry, bacteriology, dairy bacteriology, chemistry, dairy chemistry and agricultural economics.

Of the veterinary colleges studied only two offered any courses in the fundamentals of milk and its products and these were of a limited nature. More courses were offered in animal husbandry, but this was due to the fact that farm animals other than cows were studied.

None of the schools of sanitary engineering offered any courses of study in either dairy manufactures or animal husbandry.

Thus, it would seem that the dairy college graduate would be the logical choice for milk sanitation work.

From a questionnaire sent to cities of more than 100,000 population, it was ascertained that in 32 out of 79 of these cities answering the questionnaire, no dairy graduates were employed in milk sanitation.

The fact that dairy graduates are being discriminated against is probably due to the fact that the dairy colleges have been too prone to overlook the public health field, concentrating their interest in turning out men for the industry; also, many health officials have become oblivious to the virtues of the technically trained dairy college graduate when milk sanitarians are being sought.

"While many milk sanitarians are sanitary engineers who by practice and experience have become expert in this particular line of endeavor, the preeminence of the educational background of the dairy graduate cannot be denied—by this virtue above all, is he (the dairy college graduate) the logical candidate for milk sanitation and all it implies." L.H.B.

55. Properties of Detergent Solutions. Thermal pH Coefficients of Alkaline Solutions. LESTER E. KUENTZEL, JAMES W. HENSLEY, AND LESLIE R. BACON, Wyandotte Chemicals Corp., Wyandotte, Mich. Jour. Indus. and Engin. Chem., Indus. Ed., *35*, No. 12: 1286. Dec., 1943.

This is the fourth of a series of papers concerned with the properties of detergent solutions, especially those used in laundry practice. Detailed pH data at 25°, 40°, and 60° C. are presented for distilled water solutions of nine commercial alkalies sometimes used alone and in combination as soap builders. The alkalies examined were sodium hydroxide, sodium carbonate, sodium bicarbonate, trisodium phosphate, tetrasodium pyrophosphate,

sodium tetraphosphate, sodium metasilicate, sodium sesquisilicate and sodium orthosilicate. B.H.W.

56. Mixed Calcium Salts of Soaps and Anionic Detergents. GILBERT D. MILES AND JOHN ROSS, Colgate-Palmolive-Peet Co., Jersey City, N. J. Jour. Indus. and Engin. Chem., Indus. Ed., *35*, No. 12: 1298. Dec., 1943.

Mixed salts of calcium with fatty acids and synthetic anionic detergents were found to form when mixed in solution. Study of formation of the salts in mixtures containing sulfated detergents, soap and calcium salt permitted appraisal of decrease in foaming and detersive properties of the solutions. No corresponding behavior was found for magnesium salts. B.H.W.

57. Labor Saving Through Farm Job Analysis. I. Dairy Barn Chores. R. M. CARTER. Vt. Agr. Expt. Sta. Bul. 503. 66 pages, illus. June, 1943.

A detailed record was made of the time taken, the distance walked, and the routes traveled by the owner in doing the barn chores for his 22-cow dairy. After careful study of the problem a series of changes, designed to make the work easier and to save time, were made. These changes were of four general types: (1) Rearrangement of the stable; (2) Improvement of work routines; (3) Provisions of adequate and suitable equipment; (4) Convenient location of tools and supplies. As a result, the time spent on chores was reduced from 5 hours 44 minutes to 3 hours and 39 minutes daily, a saving of 2 hours 5 minutes, and the travel was reduced from 3¼ to 1¼ miles daily, a saving of 2 miles. Two hours a day is equivalent to more than 60 12-hour days, a good 2 months work, in a year; 2 miles daily is equivalent to 730 miles yearly. The money cost of the changes made was small. What this man did can be done by any dairy farmer who will undertake the task seriously. Many of the ideas worked out on his farm can be applied on other farms without change. In other cases some modification may be necessary to make them workable. In still others new schemes may need to be devised to solve particular problems. But the method here used of observing the chores, studying the problem, and working out improvements can be applied anywhere. Specific suggestions for the job are given; also a score card for dairy barn layout.

38 figures, 22 tables. J.G.A.

The SIXTH *of a series discussing how Nature and Man together develop our finer foods.*

IT PUTS THE "Oomph" IN MANY FLAVORS

Ask a food expert to give an example of food processing which requires getting rid of a lot of material in order to recover the *wanted* product and he is quite likely to think of sugar. It is a good example.

If you go on to the next type of processing and ask for an example wherein something has to be *added* to get results, the chances are he will think of citric acid as that "something"—the acid that puts the "oomph" in many flavors.

In various combinations, citric acid has had an important place in diet and food processing from 'way back. Seventy-five years ago, every British ship captain was required to give each member of his crew an ounce of lemon or lime juice every day. Although it was unknown at the time, these juices supplied the men with vital vitamin C. But it is largely the tang of the citric acid content which makes these juices so palatable.

Today, citric acid plays many roles. When the demand exceeded the amount which could be taken from the world crop of limes and lemons, the laboratory came to the rescue and produced citric acid by synthesis. Now, most of the citric acid used in the food and allied industries is the synthetic product made by modern chemical plants.

This versatile acid, which is so invigorating to the taste, is important as a flavor in itself. But the point of particular interest in this discussion is where it is so useful in supplying tartness to bring out the taste of other flavors.

Citric acid has the ability to make canned string beans taste more like string beans . . . and artichokes taste more like artichokes. Then again, in ices and sherbets, this "backer-up" can make pineapple taste more pineapple . . . raspberry taste more raspberry . . . and blackberry taste more blackberry.

It all comes about through the particular action upon the human taste apparatus. Our taste just seems to respond when certain things take place in the mouth—*no action, no taste.*

Therefore, the processor, when dealing with flavor, has to know a lot about taste sensations.

In addition to the problems of flavor-loss in freezing or baking, he has to know what is necessary to get the taste organs ready to record delicate flavors. He can't just squeeze a raspberry, for instance, and try to take refuge in the fact that it is all raspberry. It might be all raspberry at the start, but by the time it has gone through all the steps in making the final product, it has lost much of its natural taste-thrill. It is the processor's job to know what to do to deliver the finest flavor to the ultimate consumer.

Long study and experience have proved that additions to the basic ingredients in many cases are necessary to produce a true-to-life flavor at the time of consumption. Just as citric acid gives character to certain fruit flavors, added vanillin is necessary to go along with the vanilla bean in order to achieve a full vanilla flavoring at the time the ice cream or other food product reaches the consumer's plate.

It's something like aerial gunnery—***you have to shoot for where the plane isn't, so you'll hit the target when it gets there!***

Published in the interest of continued progressive thinking in the food industries, by

DAVID MICHAEL & CO.

makers of Mixrom and other powdered vanilla products

Front and Master Streets, Philadelphia 22, Pa.

Your advertisement is being read in every State and in 25 Foreign Countries

Acres of diamonds—FOR DAIRYMEN!

PUT 'em all together and they spell general dairy equipment cleaning the speedy and *thorough* way—with Wyandotte G.L.X.!

This dairy cleaning favorite makes a specialty of working with water at its hardest . . . on short-time plate heaters . . . on glass, monel, stainless steel and other metal surfaces—*safely*.

Remember—Wyandotte has the *complete* line of materials available not only for all phases of plant cleaning, but also for bottle washing, germicidal treatment and cream acidity standardizing for butter manufacture.

FOR YOUR DAIRY FARMERS:

Packaged conveniently for dairy farm use are the Wyandotte Products they especially need! Among them:

- G.L.X.—The sudsy, all-around dairy utensil cleaner in 2-lb. packages.
- WYANDOTTE CLEANER and CLEANSER—Makes no suds; used with water that's soft or average hard.
- WYANDOTTE STERI-CHLOR—Potent germicide for use after cleaning. It destroys bacteria. It's safe on metal surfaces and rubber.

Wyandotte

REG. U. S. PAT. OFF.
Service Representatives in 88 Cities

WYANDOTTE CHEMICALS CORPORATION · J. B. Ford Division · Wyandotte, Michigan

Your advertisement is being read in every State and in 25 Foreign Countries

EVERYTHING YOU NEED FOR SEDIMENT TESTING

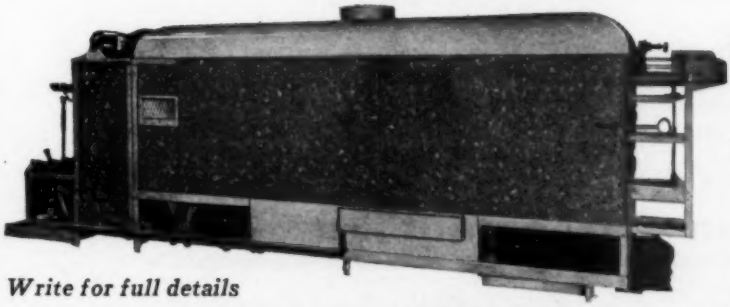
"E–Z LOCK" Sediment and Mold Test Cards
"Credicott" Milk, Cream and Mold Discs
"Langsenkamp-Wheeler" Milk Sediment Testers
New "Illinois" Cream Sediment Testers

SEDIMENT TESTING SUPPLY CO. MILT. KING Exclusive Distributors
20 E. Jackson Blvd., CHICAGO 4, ILL.
"World's Largest Distributors of Sediment Test Cards"

I'll take Milk!

225° Sterilization Assured with
The **L-P CONSERVATION CAN WASHER**
and **MIKRO-SAN ACID CLEANER**

Wash Water 150°—Pump Rinse Water 225°
Sterile Rinse Water 225°



PREVENTS THE DEPOSIT OF LIME SCALE AND MILKSTONE IN CANS.
—
Reduces bacteria to practical sterility.

Write for full details

LATHROP-PAULSON COMPANY
2459 W. 48th St., CHICAGO 32 154 W. 42nd St., NEW YORK 18
2261 15th Ave., SAN FRANCISCO 16

Your advertisement is being read in every State and in 25 Foreign Countries

Why Patapar is called the TWO-PURPOSE PAPER

As a wrapper and packaging material, Patapar* Vegetable Parchment offers two unique features.

Strong when wet— Soak Patapar in water and it will remain firm and strong. It can be *boiled* without harm.

Resists grease—Patapar is proof against grease, fats, oils. Smear some on it and you'll see how it resists penetration.

Because of this double-duty characteristic, Patapar has been able to solve many difficult packaging problems. And it lends itself to artistic printing. It is the ideal material for:

BUTTER WRAPPERS	MILK CAN GASKETS
TUB LINERS AND CIRCLES	MILK BOTTLE HOODS
PRINTER BOX LINERS	CHEESE WRAPPERS AND LINERS
BULK CORRUGATED BOX LINERS	ICE CREAM FLAVOR LABELS

And many other uses

*Reg. U.S. Pat. Off.

Paterson Parchment Paper Company

Headquarters for Vegetable Parchment since 1885

Bristol, Pennsylvania

WEST COAST PLANT: 340 BRYANT STREET, SAN FRANCISCO 7, CALIFORNIA
BRANCH OFFICES: 120 BROADWAY, NEW YORK 5, N. Y. • 111 WEST WASHINGTON STREET, CHICAGO 2, ILL.

Your advertisement is being read in every State and in 25 Foreign Countries

SCIENCE and SALT

The salt industry of the U. S. is,—as time goes,—still a comparatively new industry.

Less than sixty years ago salt was a pretty hap-hazard product. The principal virtue of salts that were called butter salt and cheese salt then, was that they came out of a sifting apparatus between screen mesh number so and so and number so and so.

There had been no start at chemical refining; and solubility hadn't even been thought of. In the language of those days, "Nature takes care of things like that."

When we examine CHIPPEWA TISSUE FLAKE SALT today, with its chemical purity of over 99.90 per cent and other characteristics of utmost perfection, it is hard to believe that a product, intended for similar uses, could ever have been as crude as salt was, not too many years ago.

Of course, the answer is science. It was science that pointed the way to perfection in the four essentials of CHIPPEWA Butter Salt and Cheese Salt, viz.: chemical purity, quick, perfect solubility, superb cleanness and mild flavor.

There is a type of CHIPPEWA SALT made to produce best results in every kind of dairy product.

A liberal sample for you to check is yours for the asking.

THE OHIO SALT COMPANY
WADSWORTH, OHIO
H. E. SCHUKNECHT, Mgr., Dairy Division
308 W. Washington St. Chicago, Ill.

TISSUE FLAKE

Your advertisement is being read in every State and in 25 Foreign Countries

FOOD PLANT SANITATION

An invitation from The Rex Company to acquaint you with the latest methods in food plant sanitation.

MIKRO-SAN

U.S. Pat. No. 2,338,688

The original acid cleaner for can washing and general cleaning.

In can washing MIKRO-SAN results in cleaner cans and prevention of milkstone formation with less expense than other cleaners.

MIKRO-SAN gives excellent results on such cold equipment as milk or cream holding vats, ice cream freezers, etc. The amount necessary to use is governed by the degree of water hardness and contamination.

REX MILKSTONE REMOVER FOR TINNED EQUIPMENT

U.S. Pat. No. 2,338,688

Formerly known as Formula 1029-1. The safe cleaner for removing milkstone and waterstone from all tinned surfaces.

Removes all milkstone deposits from cheese hoops, tinned coils, etc., and leaves a bright, shiny, sweet-smelling surface. The strength of solution necessary varies depending upon the length of the soaking period.

REX MILKSTONE REMOVER FOR STAINLESS STEEL

U.S. Pat. No. 2,338,688

An extremely active and effective cleaner for such difficult jobs as deodorizers, plate pasteurizers, etc.

This milkstone remover when used in the proper proportion will remove and prevent the accumulation of milkstone on all stainless steel surfaces.

REXCO-PHOS

Formerly known as No. 372-2. A balanced alkali compound in globular form containing complex phosphates, wetting agents, etc.

A safe and active cleaner for general use whenever a good alkali cleaner is needed. When used in the proper proportion it will keep the hard water salts in suspension and remove all the fat and grease from the equipment without leaving a film.

REX ACID INHIBITOR

Makes possible the descaling of bottle washing and can washing machines safely and effectively with no danger of freezing or sticking the machine.

Prevents excessive acid fumes, protects the metal and at the same time lubricates the machine.

EXCELCIDE VAPO SPRA

Method of cockroach and insect control. EXCELCIDE VAPO SPRA is a specially fortified spray that kills insects by inducing both a respiratory and a leg paralysis.

EXCELCIDE REPEL MIST

A non-poisonous insect repellent. Kills insects by leg paralysis when applied around congregating points or breeding places.

Only periodic applications are necessary.

DOW METHYL BROMIDE

Gas fumigation. METHYL BROMIDE fumigation is a sure and positive means of eradicating all insect and rodent infestations. This gas kills all stages of insect life from the egg to the adult and is just as lethal for all species of rodents.

The Rex Company will either contract to fumigate the plant or supply the necessary materials for a fumigation.

CAUTION—This gas is lethal for man and should only be used under expert supervision.

A booklet describing in detail all of the above sanitary products and the methods of application will be sent upon request.

THE REX COMPANY—BURLINGTON, IOWA.

Your advertisement is being read in every State and in 25 Foreign Countries

FOR ALL DAIRY SANITATION

B·K REG. U.S. PAT. OFF. CHLORINE BACTERICIDE

Every year, because of poor quality, enough milk and milk products to meet the annual nutritional needs of 120,000 soldiers are wasted. This statement, issued by a University of Illinois College of Agriculture authority, calls for action.

For *fast* action, the answer is B-K Chlorine Bactericide . . . in up-to-the-minute dairies and creameries. Its available chlorine goes right to work as a sanitizer. Efficient and rapid, it costs little to use. Use B-K daily as a sanitizing rinse, and a helpful shortcut to increased butter production . . . through decreased rejections.

Dept. DS, B-K Division
PENNSYLVANIA SALT MANUFACTURING COMPANY
Chemicals
1000 WIDENER BUILDING, PHILADELPHIA 7, PA.

ACCEPTED BY PUBLIC HEALTH AUTHORITIES

Mention JOURNAL OF DAIRY SCIENCE When Answering Advertisements

This helps both the Advertiser and you

"HANSEN'S"
Dairy Preparations

Cheese Rennet and Color
Annatto Butter Color
Certified Butter Color
Ice Cream Color
Lactic Ferment Culture
Bulgarian Culture
—★—
Cheese Bandages, Circles
Press Cloths
Odorless Dairy Fly Spray
Testing Solutions
Rennet Tests

CHR. HANSEN'S LABORATORY, INC.
MILWAUKEE, WISCONSIN

Your advertisement is being read in every State and in 25 Foreign Countries

AMERICAN DAIRY SCIENCE ASSOCIATION

INCORPORATED IN THE DISTRICT OF COLUMBIA

Officers

President	A. C. DAHLBERG, Ithaca, New York
Vice-President	A. C. RAGSDALE, Columbia, Missouri
Secretary-Treasurer	R. B. STOLTZ, Columbus, Ohio
Editor	T. S. SUTTON, Columbus, Ohio
Director	P. H. TRACY, Urbana, Illinois
Director	C. L. BLACKMAN, Columbus, Ohio
Director	R. B. BECKER, Gainesville, Florida
Director	W. E. PETERSEN, St. Paul, Minnesota
Director	G. MALCOLM TROUT, East Lansing, Michigan
Director	J. C. KNOTT, Pullman, Washington
Director	H. P. DAVIS, Lincoln, Nebraska

Officers of Sections

DAIRY PRODUCTION SECTION

Chairman	DWIGHT L. ESPE, Ames, Iowa
Vice-Chairman	G. W. SALISBURY, Ithaca, New York
Secretary	D. M. SEATH, University, La.

DAIRY MANUFACTURING SECTION

Chairman	PAUL F. SHARP, San Francisco, Calif.
Vice-Chairman	L. K. CROWE, Lincoln, Nebraska
Secretary	I. A. GOULD, East Lansing, Michigan

DAIRY EXTENSION SECTION

Chairman	E. C. SCHEIDENHELM, East Lansing, Mich.
Vice-Chairman	FLOYD ARNOLD, Ames, Iowa
Secretary	GERALD HEEBINK, Morgantown, W. Va.

Officers of Divisions

SOUTHERN

Chairman	R. B. BECKER, Gainesville, Fla.
Vice-Chairman	J. P. LAMASTER, Clemson, S. C.
Secretary-Treasurer	F. H. HERZER, State College, Miss.

EASTERN

Chairman	H. C. MOORE, Durham, N. H.
Vice-Chairman	A. I. MANN, Storrs, Conn.
Secretary-Treasurer	L. R. DOWD, Storrs, Conn.

WESTERN

Chairman	N. S. GOLDING, Pullman, Wash.
Vice-Chairman	S. W. MEAD, Davis, Calif.
Secretary-Treasurer	H. P. EWALT, Corvallis, Oregon

The American Dairy Science Association was organized to advance the general welfare of the dairy industry, especially by the improvement of dairy instruction by the stimulation of scientific research in all phases of the subject and by improvement in methods of conducting extension work.

Membership. Any person is eligible to membership who is formally announced by an Agricultural College or Experiment Station, or by the Bureau of Dairy Industry of the United States Department of Agriculture or by the Canadian Department of Agriculture as an instructor, extension worker, investigator, or administrative officer connected with the dairy industry, or any person filling a position of responsibility connected with the dairy industry who has had a college or university training in technical science, or any person filling a responsible position in the dairy industry of a professional character requiring a technical knowledge of dairying of a high order. The membership fee is $5.00.

The dues are $5.00 a year, $3.00 of which is for a year's subscription to the *Journal of Dairy Science*. Correspondence regarding membership and dues should be addressed to R. B. Stoltz, Ohio State University, Columbus, Ohio.

FREE TECHNICAL BULLETINS

Compiled by the
DIVERSEY RESEARCH LABORATORIES

THE CONTROL OF PIN POINT BACTERIA

Summary of Contents

Why called "pin point" bacteria . . . thermoduric bacteria . . . thermophilic bacteria . . . source of pin point bacteria . . . control on the farm . . . control in the plant . . . disinfecting of milk cans . . . why re-pasteurization should be avoided . . . dead ends . . . foam . . . refrigeration of pasteurized milk . . . importance of milkstone control . . . disinfecting of plant equipment.

Diversey Technical Bulletins have been prepared in the interest of better sanitation in the nation's dairy, beverage, and other food plants. Copies are available without cost or obligation. Simply address your request to The Diversey Corporation, 53 W. Jackson Blvd., Chicago.

WIN THE WAR WITH FOOD

NEED HELP? JUST CALL A DIVERSEY D-MAN

FLAV-O-LAC FLAKES

THE CULTURE

of definitely better flavor & aroma-producing qualities.

The standard with foremost operators, agricultural schools & colleges.

FLAV-O-LAC FLAKES (shown) produce a quart of the finest starter on a single propagation. Single bottles $2.00.

SPECIAL FLAV-O-LAC FLAKES "40"

produce 40 quarts of starter on a single propagation. Single bottles $3.00

Free Culture Manual of Fermented Milk Products on request.

Pioneers in Spectro-chemical, Chemical and Fluoro-photometric Determinations of Vitamins A, B_1, B_2, Nicotinic Acid, Pantothenic Acid, B_6, C & E in Dairy and Food Products. (Vitamin D excluded) inquiries invited.

THE DAIRY-LABORATORIES

A DIVISION OF DALARE ASSOCIATES

23rd & Locust Sts., Phila., Pa.

BRANCHES

New York Baltimore Washington

See our catalog in Dairy Industries Catalog.

How to Step-Up Can Washing Efficiency

Do you know how an increasing number of dairies, creameries, milk and ice cream plants are overcoming hard water conditions in their can washing? By using that specialized detergent.

OAKITE COMPOSITION No. 82

For it has a dual purpose: Because of its vigorous yet safe cleaning action, it gives you sanitary, odor-free cans at low cost . . . because of its unique lime-solubilizing characteristics, it retards lime-scale formation in lines, jets and spray heads. Write for details.

OAKITE PRODUCTS, INC.,
Dairy Research Division,
16G THAMES STREET, NEW YORK 6, N. Y.

Technical Service Representatives Located in All Principal Cities of the United States and Canada

OAKITE Your Friend for Cleaning CLEANING

Your advertisement is being read in every State and in 25 Foreign Countries

Sometimes, better formulas are written with an eraser!

Most great products are the result of ceaseless experiment, improvement and change. Yet, how often is all chance of improvement brushed aside with the casual remark—"We don't like to change our formula"? Too frequently, the same old formula has been used so long that even the idea of changing it is seldom considered. But "the same old formula" isn't always the best formula. Vanilla, for example, is such an important ingredient and plays such a big part in the ultimate popularity and sale of any product that nothing less than the best can ever be good enough. Yet, how long is it since you've even questioned the vanilla you are now using?

If you are not one of the many ice cream manufacturers already using Veritivan, why don't you try it and see how much rich, delicious goodness it will add to your own ice cream? We think you'll agree with so many others that Veritivan is truly the best of all fine flavors.

FOOD MATERIALS

CORPORATION

Manufacturing Flavor Chemists

2521 WEST 48TH STREET • CHICAGO

Branch Offices in all Principal Cities

VERITIVAN

Food Materials Corp.
2521 W. 48th St., Chicago, Ill.

Please send 1 lb. Free Sample of Veritivan

Company_____

By_____

City_____ State_____

Copies of the 20-Year Index covering Volumes I to XX, inclusive are available at the following prices:

MEMBERS

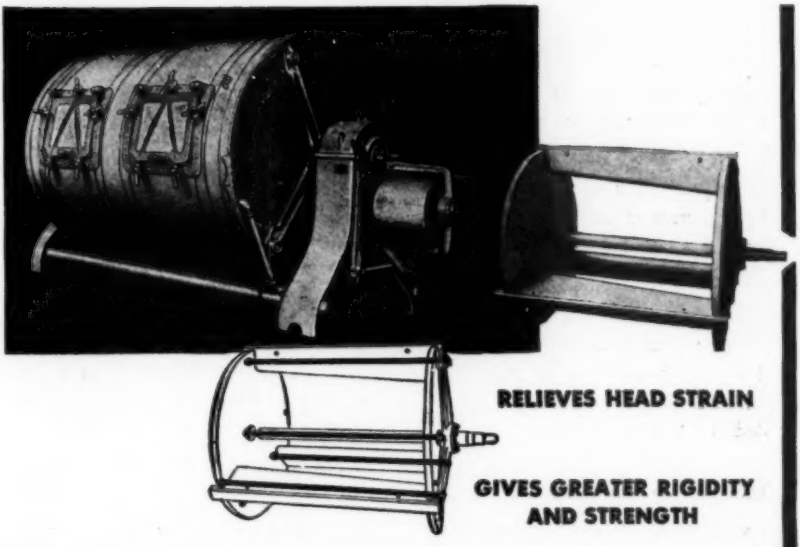
Cloth Bound	**$2.35**
Paper Bound	**2.00**

NON–MEMBERS

Cloth Bound	**$5.50**
Paper Bound	**5.00**

Your advertisement is being read in every State and in 25 Foreign Countries

CP's *Exclusive* SEALED-IN TIE-ROD CONSTRUCTION



RELIEVES HEAD STRAIN

GIVES GREATER RIGIDITY AND STRENGTH

WHEN you choose a CP Sanitary No-Roll Churn with its exclusive sealed-in tie-rod features, you get the strongest and most sanitary construction possible. Note from illustration above how the tie-rods—entirely sealed away from churn interior — extend through the shelves and are fastened outside of the gudgeon arms. Center working bar also has the sealed-in tie-rod feature.

Unexcelled performance — the tapered shelves and center working bar work the product the full length of drum and provide uniform composition.

Buttermakers like the fact that with the CP No-Roll Churn they can work the butter hard or soft without its getting sticky . . . obtaining a dry, firm waxy textured butter which GIVES MAXIMUM YIELD WHEN PRINTED. Ask for Bulletin K-689.

THE CREAMERY PACKAGE MFG. COMPANY, 1243 W. Washington Blvd., Chicago 7, Ill.
Branches: Atlanta—Boston—Buffalo—Chicago—Dallas—Denver—Kansas City
Los Angeles — Minneapolis — New York — Omaha — Philadelphia — Portland, Oregon — Salt Lake City — San Francisco — Seattle — Toledo — Waterloo, Iowa
CREAMERY PACKAGE MFG. CO. OF CANADA, Ltd., 267 King St. West, Toronto, Ont., Canada

4TH WAR LOAN

DIFCO

CULTURE MEDIA
for Examination of Milk

Bacto-Tryptone Glucose Extract Agar

is recommended for use in determining the total bacterial plate count of milk in accordance with the procedures of "Standard Methods for the Examination of Dairy Products" of the American Public Health Association.

Upon plates of medium prepared from Bacto-Tryptone Glucose Extract Agar colonies of the bacteria occurring in milk are larger and more representative than those on media previously used for milk counts.

Bacto-Proteose Tryptone Agar

is recommended for use in determining the bacterial plate count of Certified Milk. The formula for this medium corresponds with that suggested in "Methods and Standards of Certified Milk" of the American Association of Medical Milk Commissions.

Bacto-Violet Red Bile Agar

is widely used for direct plate counts of coliform bacteria. Upon plates of this medium accurate counts of these organisms are readily obtained.

Bacto-Brilliant Green Bile 2% and
Bacto-Formate Ricinoleate Broth

are very useful liquid media for detection of coliform bacteria in milk. Use of these media is approved in "Standard Methods."

Specify "DIFCO"

THE TRADE NAME OF THE PIONEERS
In the Research and Development of Bacto-Peptone and Dehydrated Culture Media

DIFCO LABORATORIES
INCORPORATED
DETROIT, MICHIGAN